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PiRamid: A compact Raspberry Pi imaging box to automate small-scale time-lapse digital analysis, suitable for laboratory and field use

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ABSTRACT

Digital imaging permits the quantitation of many experiments, such as microbiological growth assays, but laboratory digital imaging systems can be expensive and too specialised. The Raspberry Pi camera platform makes automated, controlled imaging affordable with accessible customisation. When combined with open source software and open-source 3D printed hardware, the control over image quality and capture of this platform permits the rapid development of novel instrumentation. Here we present "PiRamid", a compact, portable, and inexpensive enclosure for autonomous imaging both in the laboratory and in the field. The modular three-piece 3D printed design makes it easy to incorporate different camera systems or lighting configurations (e.g., single wavelength LED for fluorescence). The enclosed design allows complete control of illumination unlike a conventional digital camera or smartphone, on a tripod or handheld, under ambient lighting. The stackable design permits rapid sample addition or camera focus adjustment, with a corresponding change in magnification and resolution. The entire unit is small enough to fit within a microbiological incubator, and cheap enough (~£100) to scale out for larger parallel experiments. Simply, Python scripts fully automate illumination and image capture for small-scale experiments with an ~110×85 mm area at 70–90 μm resolution. We demonstrate the versatility of PiRamid by capturing time-resolved, quantitative image data for a wide range of assays. Bacterial growth kinetics was captured for conventional microbiology (agar Petri dishes), 3D printed custom microbiology labware and microfluidic microbiology. To illustrate application beyond microbiology, we demonstrate time-lapse imaging of crystal growth and degradation of salad leaves. Minor modifications permit epi-illumination by addition of a LED ring to the camera module. We conclude that PiRamid permits inexpensive digital capture and quantitation of a wide range of experiments by time-lapse imaging to simplify both laboratory and field imaging.

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Specifications table

Hardware name	PiRamid (Pyramid Imaging Rig)
Subject area	• Microbiology • Environmental science • Agriculture Science • Chemistry • Crystallography and crystal engineering
Hardware type	• Imaging tool • Quantitative imaging
Closest commercial alternative	Gel documentation and laboratory imaging boxes
Open Source license	CC-BY 4.0 Creative Commons Attribution-ShareAlike 4.0
Dimensions of hardware	190 mm (L) × 140 mm (W) × 140 mm (H) (incl. Raspberry Pi)
Cost of hardware	£107.98 (£158.97 incl. touchscreen and UPS)
Source file repository	Digital Object Identifier DOI https://doi.org/10.5281/zenodo.7090151URL : https://zenodo.org/record/7090151

Hardware in context

Many current standard laboratory methods and assays can benefit from digital photography to capture, quantify, and digitise results but this can be time-consuming and not automated without dedicated imaging systems. While some commercial systems exist for specific common applications, such as electrophoresis gel documentation, these can be expensive and are typically tailored to particular setups (e.g., UV or blue-fluorescent illumination). This limits digital imaging to particular assays, the cost can be a barrier to access, and large expensive benchtop instruments are unsuited to carry outside of the lab and into the field. The number of uses of digital imaging - often exploiting consumer products such as smartphone cameras is rapidly expanding within many biological and chemical sciences. For example, smartphone colourimetry [1], illustrates how analysis of spectral changes in reporter dyes used widely in biological and chemical assays, can be transported away from spectrometers, imaging processing software quantify colour from RGB digital images and convert them into absorbance values. Within microbiology, colourimetry and turbidity can be used to detect and measure bacterial growth. Likewise in chemical analysis [2], where once semi-qualitative approaches to comparing colour change by eye can be replaced with more sophisticated and quantitative image analysis using digital imaging. The development of Digital Image Correlation (DIC) techniques is becoming popular in fields including crystallography [3]. As digital cameras become cheaper, a move from laboratory instruments (e.g. CCD cameras) to consumer cameras offers a simplified approach to producing scientific imaging devices, remaining sensitive yet cost-effective in a resource-limited setting, from chemical sensors [4] to phenotypic identification of bacteria [5]. Often, however, data quality is restricted by manual camera operation to take images with a digital camera or smartphone camera. Although the use of a tripod or frame to support the camera can stabilise image capture, consumer cameras with proprietary control firmware can be tricky to control offering limited or unreliable time-lapse options. Automation of these processes can therefore greatly increase the number and quality of images recorded and reduce the hands-on time needed to take the images. There have already been several advances across different fields highlighting how automation reduces time in the field and allows for the preservation of data integrity [6,7]. Another trend is toward portability, to take lab measurements outside the lab and into the field [1]. Indeed, the increasing popularity of smartphone capture for many diverse assays [8,9] suggests that the rapid development of customisable, precise imaging devices will offer a portable format for experimental imaging in many experimental fields.

Screening and imaging are often found within clinical microbiology labs, but although automation has been extensively adopted in clinical diagnostic labs, it remains expensive and/or laborious in smaller labs or research areas that do not use the core standardised assays required in clinical testing [10,11]. Here we introduce why microbiology methods are important, and yet outside the best-funded clinical labs, limited instruments are available, and flexibility is key. Due to the specialism of many microbiological samples, and the range of different ways in which experiments are carried out, customisable imaging and analysis systems are vital. A range of different sample formats may be required for analysis, even when core methods remain the same. For example, testing mastitis milk samples in an agricultural setting will require a distinct set of analytical microbiology assays to identify and test antibiotic susceptibility of bacterial pathogens, to human clinical samples. Yet in both cases, core assay equipment, multi-well plates, and Petri dishes remain identical requiring quantitation of colour changes that indicate microbial identification, in a range of different devices and conditions. Recent innovations have shown that traditional large devices can be replaced by smaller devices including custom 3D printed labware [12] down to the smallest sample volumes being assessed within microfluidic devices [13,14] and droplet microfluidics [15,16]. Flexibility and customisation are therefore vital for a lab imaging platform to be useful for as many different applications as possible. One of the most important analytical microbiology methods is the identification of antimicrobial resistance (AMR) in a wide range of samples. Currently, these assays either require skilled laboratory technicians or expensive automated instrumenta-

tion, both of which are operated within an appropriate lab environment. This highlights that simplicity in the way we image and analyse assays is required outside of larger-scale labs where funds are extensive, and the sample throughput is high.

When addressing problems such as AMR on farms, treatment is almost always carried out before microbiological testing, days before AMR would be identified. This historically has contributed to a continual build-up of AMR on farms due to the overuse or inappropriate use of antibiotics [17,18]. A more flexible and rapid approach is required to tackle these problems. Flexible time-resolved automated imaging could therefore become important in agriculture, for example supporting the development of more rapid AMR tests ideally measuring bacterial resistance to antibiotics directly from the sample. For use in the dairy industry, it would be ideal to analyse milk samples from dairy cows with mastitis for antibiotic resistance. Potential use for this device would be to image antibiotic susceptibility assays in mastitis milk samples. Portable, timelapse imaging would therefore be able to determine AMR more rapidly on-farm, highlighting the usefulness of PiRamid outside of the lab. Moreover, the importance of kinetics in microbial growth analysis has been shown by the existence of much more costly and sophisticated in-house laboratory imaging systems, however, the simplicity and automation of PiRamid may prove useful for a much quicker and simpler way of analysing AMR on dairy farms in the future.

Microfluidic devices are now more widely used in the detection of pathogenic species of bacteria, with their use allowing for greater speed of detection and the development of point-of-care diagnostics, able to be used in the field. Whether this be within the detection of urinary tract infections (UTIs) [13] or for the portable detection of bacteriophage lysis [9]. Our group developed a simple, low-cost example microfluidic device that can be used to detect bacterial growth and measure multiple antimicrobial resistance profiles of bacteria using the metabolic sensitive dye, resazurin [14] able to detect bacterial growth by a colour change from blue to pink. The devices used are made from a melt-extruded highly transparent fluorinated ethylene propylene co-polymer (FEP-Teflon) microcapillary film (MCF) comprising 10 capillaries along its length. This method allows for the use of high-throughput microfluidic devices, termed 'lab-on-a-comb', compatible with existing laboratory equipment such as 96 well microtitre plates [9,14,19]**.** The use of microfluidic devices can provide detailed information on bacterial growth, morphology, and kinetic effects of substances on bacterial species, which can prove beneficial to phenotypic analysis for both research and clinical applications (e.g., identify pathogens and choosing antibiotic treatment for UTIs, and AMR surveillance). Previously the reliance on expensive plate readers to collect this data was time-consuming and labour-intensive, replacing plate-readers with time-lapse cameras could easily increase throughput, reduce labour time and increase flexibility since different formats can be tailored to specific needs.

To address this, we have seen the development of open-source imaging devices, exploiting the use of 3D printing and utilising robotics [6,19]. Devices such as the one described by Needs et al. [19] allow for fully customisable and high throughput imaging of both low-cost microfluidics and conventional MTP and Petri dishes. Others have developed open-source hardware to obtain the same outputs at lower cost, or when proprietary commercial instruments are not suited to novel configurations [20–22]. These could replicate or improve on established analytical microbiology systems based on reagent-loaded 96 wells plates, including those for kinetic analysis of single plates or up to 50 microtitre plates, which are monitored every 15 min [23]. The success of these commercial devices proves the value of kinetic microbial growth analysis, but the instrumentation is not widely available to most labs due to cost and specialism of experiment format, as only a few labs process enough plates to justify the capital investment in this dedicated instrument.

We propose "PiRamid" an imaging system that was designed to exploit low-cost desktop fused-filament deposition (FFD) 3D printing and simple python script programming to produce a compact, low-cost, high-performance system for automated laboratory imaging. The design centres around the simple to use and low-cost Raspberry Pi single-board computer system and associated camera. LED sample illumination is powered by GPIO pins. The system is controlled by basic Python scripts based on the widely documented PiCamera camera control library. The device is fully customisable, with the 3D printed case stacking for ease of opening, designed using OpenSCAD open-source CAD software. This design is compact and can be portable for use in the field, whilst maintaining the same automation as when used in the laboratory if powered using inexpensive consumer lithium battery packs sold typically as smartphone chargers. To make it compact, the device is scaled down to allow for easier transportation and storage; this does limit the imaging area and restricts the number of samples that can be simultaneously imaged. Importantly, the small size allows it to be used inside standard table-top microbiological incubators for controlled temperature, without requiring either a large incubator facility or built-in heading and temperature control. Although open-source incubator designs exist, and low-cost PID controlled incubators can maintain a suitable temperature for microbiological experiments [24] we avoided adding an incubator to the PiRamid to make it as simple to assemble and program as possible.

The simple system is capable of taking time-resolved images of samples of different microbiological based assays that can be performed in microfluidic devices, strip wells, and custom agar device designs. We provide validation data of its uses within different scientific fields and across different methods. With the use of microfluidic MCF, PiRamid can image up to 24 different bacterial isolates, with up to 240 different conditions (10 capillaries per isolate). Due to the ease of production and use, it would be more than appropriate to build and use multiple devices for larger experiments. This would still be cost-effective, more flexible in use and provide greater portability. Even with the average cost of an individual device ranging from ~£120 to ~£180, we can expect the production of four of these devices to cost ~£480 to ~£720, and capable of imaging up to 960 conditions. Conventional lab-based assay readers and spectrophotometers can cost far in excess of £3000 each, often analysing one individual 96-well plate at a time and incapable of capturing growth kinetics; plate readers with inbuilt incubation plus time-resolved scanning might even cost £10,000 or more, and most plate readers are too bulky to fit in microbiological incubators.

Hardware description

The PiRamid device is a simple, compact enclosure for a small CMOS camera (Figs. 1 and 2), designed for the Raspberry Pi Camera Module v2, an affordable yet high-quality CMOS image sensor with a fixed focus lens capable of producing 3280 × 2464 pixel static images. Whilst the lens has a fixed focus, this can be adjusted by screwing in or out of the casing, permitting it to be used for macro photography simply by unscrewing the lens to allow closer focus. Despite the low cost (£22 for a camera sensor with lens), this v2 camera takes excellent images and has been extensively adapted for digital microscopy [11,25,26]. Moreover, with use in incubators, producing humid environments, no issues of droplet formation within or upon the lens of the camera have been noted by users. The camera is mounted within the top section (cap) of the PiRamid and forms the lid of an interlocking 3-part pyramid of 3D printed parts. This enclosed design allows for total control of lighting for the images produced, by eliminating external ambient light. The device contains a 3D printed imaging stage that forms the bottom section of the PiRamid. The stage was designed to reduce the risk of contamination of biological materials into wiring and onto the light source and allows for easy addition and removal of samples, with the optional installation of a small knob to form a handle. All three stacking parts forming the enclosure are inexpensive and can be printed, without supports, within a standard bed of a low-cost consumer 3D printer (<200x200mm bed). The rectangular-base pyramid shape reflects the camera imaging space, and at the same time allows printing without supports since most desktop, FFD 3D printers can produce slopes of up to 45°. We printed using PLA and found no problems with mechanical or optical properties in our example applications, however, we expect the design could be printed with other filaments for example with higher melting temperatures. We and others have previously shown PLA can be sterilised using 70 % Ethanol, important for some life science applications [12,27]. We added above the imaging stage design support for a 100 × 95 mm sheet of light-scattering white translucent acrylic to make the backlight of the imaging stage more even in brightfield mode. The entire

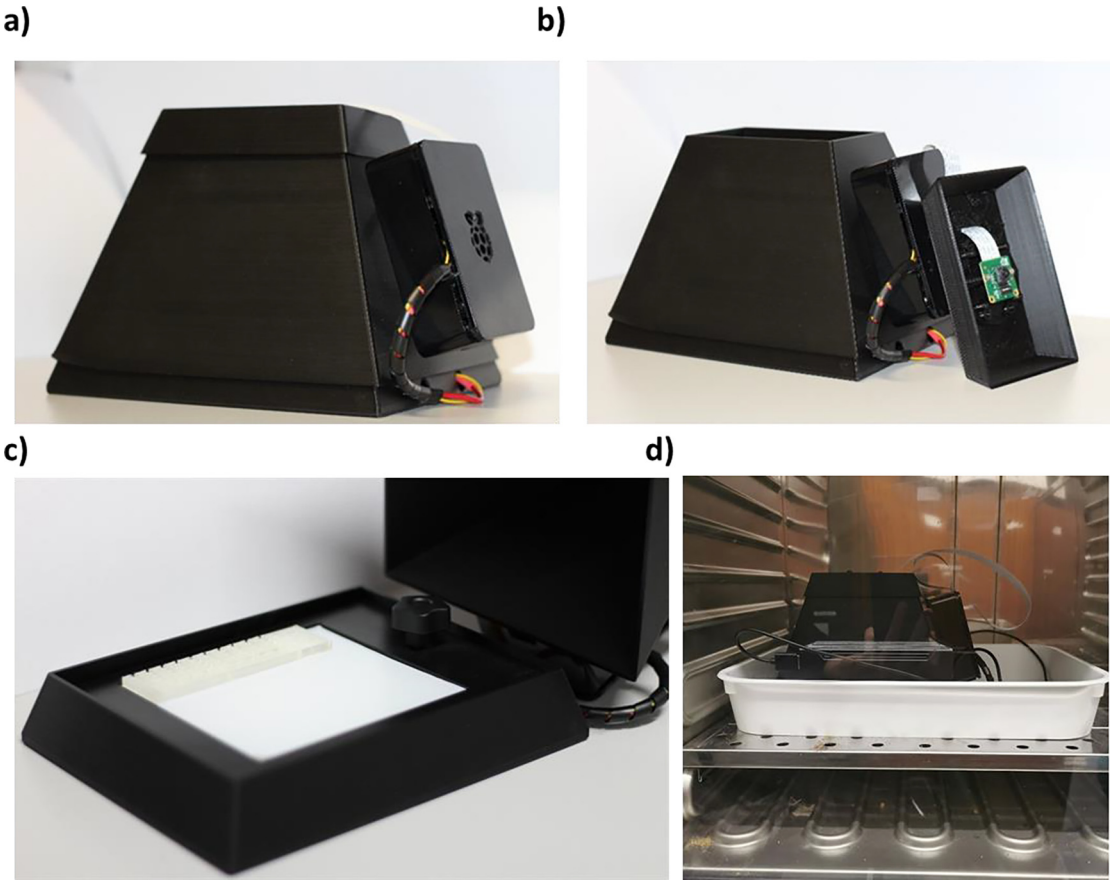

Fig. 1. Images illustrating PiRamid design, formed from three stacking 3D printed parts. (a) Compact unit in use. A Raspberry Pi computer is attached to the middle section of the box, with LED light strips wired and powered via the GPIO pins on the Raspberry Pi. (b) Raspberry Pi v2 camera is attached within the inside of the top cap section. (c) Imaging stage design. A 3D printed sample tray that slots within the bottom section of the PiRamid, preventing leakage of biological materials and for ease of samples addition. A white acrylic diffuser plate offers backlight for brightfield imaging. (d) Demonstration of compact size and portability allowing use within a tabletop incubator, powered here by an optional UPS battery unit visible in front of the PiRamid unit providing 5 V to the Raspberry Pi; external power can also be provided via USB with a power cable fed through incubator door seal.

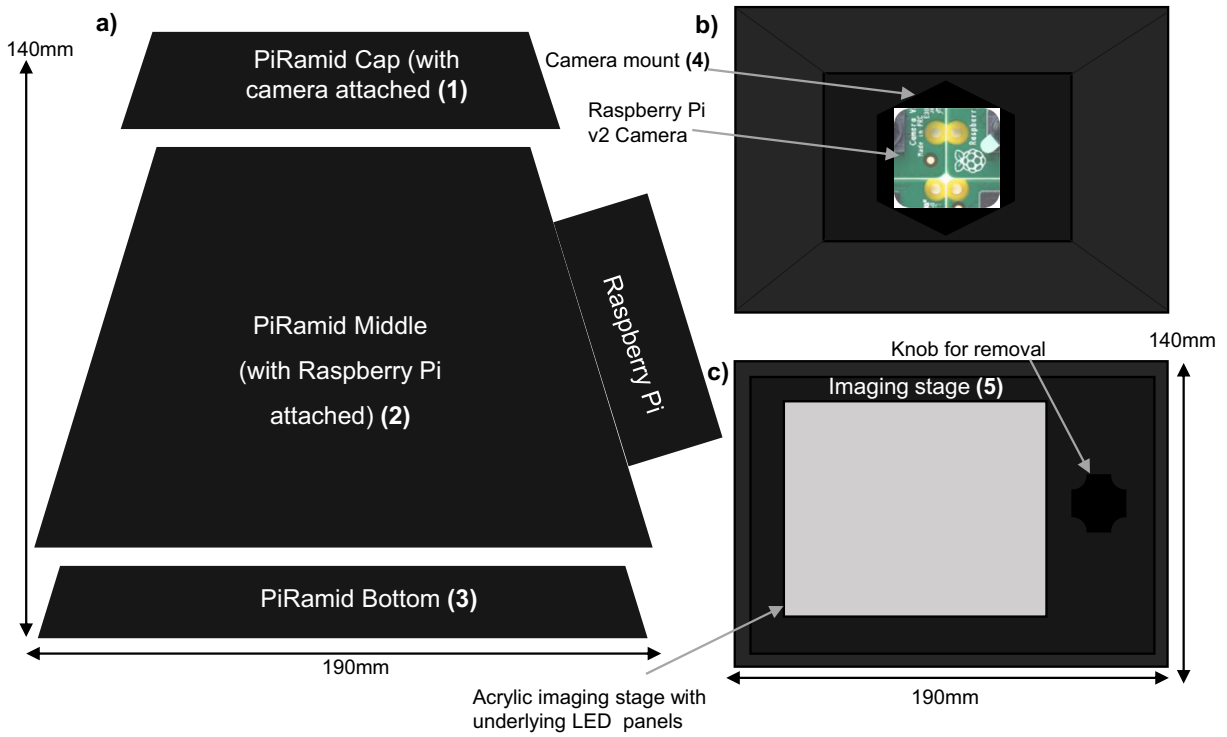

Fig. 2. Diagram of the PiRamid imaging system. (a) Side view indicates the three stacking parts with overlap between tapered section ensuring external light is blocked, with camera inside top, plus attachment of Raspberry Pi to the side with space to add a small touchscreen. (b) Inside view of the PiRamid cap demonstrating Raspberry Pi v2 camera attachment on a simple adjustable 3D printed camera mount. (c) Top view of PiRamid base tray section containing sample area and removable imaging stage with acrylic diffuser underneath which two LED light panels are placed with wiring underneath and knob for removal of sample tray.

system is controlled by a Raspberry Pi single-board computer. Two LED backlight panels are positioned in the bottom section of the PiRamid, at the base of the imaging stage, in a fixed position. These LED strips are wired to Raspberry Pi GPIO pins which are programmable to switch on and off using commands in Python script. The LED backlight units were wired with series 33-ohm resistors, drawing approximately 10 mA from the 3.3 V GPIO switched pins. The device can be operated via a remote desktop or SSH connection, which can be viewed or typed directly via a mobile phone or PC by connecting the Pi computer to WIFI or a network hotspot or using a LAN cable. PiRamid can be provided with an inbuilt screen and interface by the addition of an optional 3.5-inch touch screen which enables complete remote operation without a network connection for virtual desktop or SSH access. The device can be powered by external mains power (120–240 AC) using an inexpensive USB power supply (5 V > 2A); the addition of an uninterruptable power supply (UPS) offers greater portability (costing an additional ~£60). When required, external power (12 V adapter plugged into AC mains) can both recharge the internal UPS battery, and power the PiRamid. The USP allows the sample to be set up, Raspberry Pi started up and imaging scripts run on a desktop or near a PC, before transfer to an incubation location (e.g., inside an incubator) where it can be plugged in.

Being a fixed focus camera, the camera module lens was manually rotated to focus on the sample before use, taking care to focus on the experimental sample. A USAF 1951 high-resolution target (Edmund Optics #38-257, Edmund Optics, York UK) was used in the imaging stage when focussing to ensure the best resolution possible for the camera module. Images were taken at the centre of the imaging stage and in all four corner to compensate for distortions around the edges whilst using a stationary camera. The resolution target indicated that the resolution of the centre of the image was at or better than 7.13 lp/mm (with group 2 element 6 showing clearly separated lines), with approximately 70 μm lines spaced 70 μm apart being the smallest element visible (Fig. 3). When the resolution target was towards the corners of the image, we see reduced resolution to 5.66 lp/mm, with approximately 90 μm lines spaced 90 μm apart resolved. Even at the corners of the image, this is more than sufficient to image many targets such as microfluidic systems.

The PiRamid design mounts the camera 95 mm away from the sample, giving a field of view for samples of 116 mm × 86 mm, illuminated in a brightfield with a white backlight area of 100 × 850 mm. Note that the 3280 × 2464 pixels resolution and 116 × 86 mm corresponds to a theoretical maximum possible resolution of 35 μm per pixel, so the achieved resolution of 70–90 μm across the whole imaging area could potentially be improved somewhat for example by better focus, but not great. The achieved resolution across the whole imaging area was more than sufficient to resolve the individual microcapillaries within our microfluidic devices which are ~260 μm wide and separated by ~300-μm gaps.

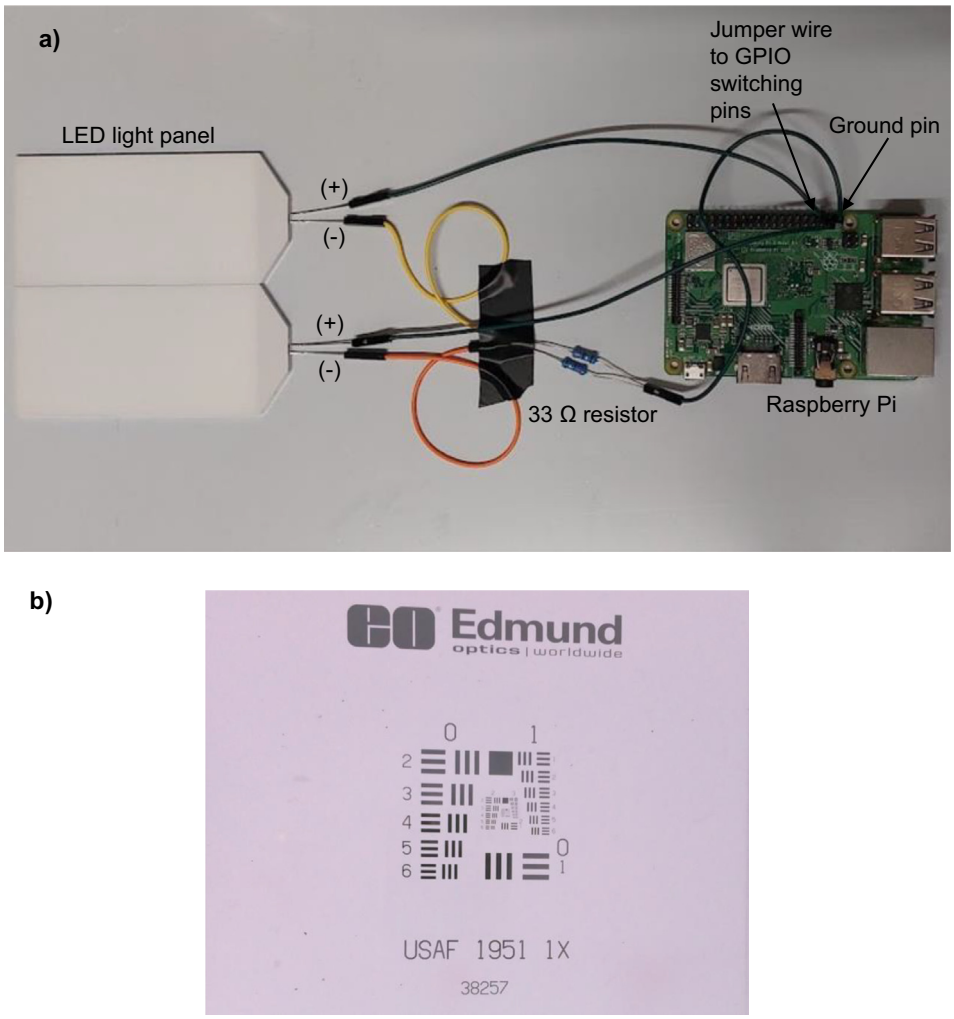

Fig. 3. (a) Wiring guide for LED light panels to Raspberry Pi (b) Demonstration of PiRamid resolution. A high resolution glass USAF 1951 resolution target was used on the imaging stage to ensure the best resolution possible for the PiCam. When using an image of the resolution target it was identified that the resolution of the image was around 70 μm with 7 lp/mm being the smallest line dimensions that could be resolved clearly (group 2 element 6 of the target).

In this current format, the PiRamid is capable of imaging one 96-well plate, one petri dish, four custom dip-slides [12], four 8-well ELISA strips, or three combs of 8 MCF strips for microfluidic testing corresponding to 240 individual 1 microliter assays (each strip contains 10 microcapillaries). The 3D printed open-source design allows for full customisation, with individual parts adaptable to varying experimental designs and required fields of view. For example, making a taller pyramid shape with a larger base section will move the camera higher, giving a larger field of view. There is a direct trade-off of resolution vs imaging area. Thus, for higher resolution images of smaller samples or fewer devices, a smaller PiRamid would work.

The system can, however, be adapted for use of Raspberry Pi's HQ camera module. This addition will allow for the capture of higher resolution images and where required, the adaptability to use with any standard C- or CS-mount lens. The HQ camera can be attached to the camera mount found in the design files. The length of the HQ camera however would require adjustment and careful lens selection would be needed as the HQ camera can be configured with different imaging angle and field of view, ranging from wide angle to telephoto; unlike the v2 camera with a single fixed lens. However, the size and shape of the PiRamids 3D structure can also be easily altered within the OpenSCAD design to accommodate a different camera distance and sample bed size, therefore making the addition of this higher budget and more sophisticated camera possible.

To take images a simple Python script was configured with the required picamera library settings (e.g. image exposure, resolution, time) and looped using Python if commands to take a specified number of images for a required length of time (the picamera library documentation is available at https://picamera.readthedocs.io/en/release-1.13/). The Raspberry Pi Foundation recently replaced the picamera library with a new camera control system following the release of "Bullseye" Raspbian operating system. Our scripts are compatible with the legacy camera control system included with the "Buster" Raspbian release. The legacy camera settings found in the previous "Buster" release can be enabled in the newer "Bullseye" release to allow for execution of our script (see Python script for further instructions). The length of time between image capture in each loop can be altered depending on the number of images required, the interval between images and the time frame of the experiment being analysed. For example, certain crystallography demonstrations require much shorter overall experiment times, with shorter imaging intervals. The images are then stored onboard the Raspberry Pi SD card and can be accessed remotely by file transfer protocol (FTP) or saved to a removable USB memory device. The Python script was also configured so that the LED strips switch on immediately before the images are taken and switch off immediately after (see design files for a text file with example script). This command is also looped alongside the imaging. Sufficient time for the camera to adjust exposure times to lighting is required after switching the LED on, alternatively, the camera exposure settings can be fixed in the script. The system was evaluated here using the Raspberry Pi's built-in VNC viewer via wireless networking to a laptop or by starting python scripts via the small touchscreen.

Main properties

1. The system is automated; the PiRamid device is capable of imaging with time intervals as short as seconds and experiment lengths up to several days.
2. The design centres around the use of simple and low-cost single-board Raspberry Pi computers, camera modules and LED backlight controlled by basic scripts using Python language; these are easy to control, and open access for customisation.
3. The device can be fully customisable, with an enclosed structure entirely consistent with low-cost desktop 3D printing, adaptable using open-source design software and enclosed to allow total control over lighting conditions.
4. This design is compact and can be considered portable for use in the field, with the addition of battery power from a UPS.
5. The device is small allowing use inside inexpensive table-top or portable incubators, for example, simple microbiology incubators, without the need for a large incubator facility or laboratory equipped with a walk-in incubator room, reducing costs, and avoiding the need for laboratory access.

Limitations

1. The device still requires the use of a tabletop or walk-in incubator for microbiological and temperature-sensitive experiments. This could be solved with a built-in incubation system to be fully compatible with field use; yet this would significantly increase.
2. The system is driven by a python script that requires some basic programming skill to modify. This could be solved with dedicated software providing a more intuitive user interface. However, most Raspberry Pi users have sufficient python familiarity to use and modify these scripts, and extensive documentation and training is available freely online, for example from the Raspberry Pi foundation.
3. As mentioned, although we can adapt the size and shape of the device, because it uses a fixed-position camera, we see a trade-off between resolution and imaging area. However, this could be addressed by customisation for the higher-cost, higher performance version HQ camera module. The device is low enough cost that multiple devices can be made to increase the area imaged or the number of samples tested per experiment.

Design files summary

The design entirely consists of customised 3D printed parts designed to stack together creating a completely closed and light-controlled environment, with the pyramidal form reflecting the minimal enclosed space for digital imaging, and the stack permitting very rapid and easy sample addition and/or camera adjustment. No glue or fasteners are required to close the device and the simple tapered overlaps between the three components eliminate external light without needing tight tolerances to ensure a close fit. We provide here the CAD design files (OpenSCAD and STL mesh) for 3D printing direct or for modification of the three main structural components to fit the user's differing needs e.g., different sample sizes, lighting configurations, or camera types. Also provided are the final STL mesh designs that can be used for 3D printing the same device configuration used in this article. This includes a camera mount design for the simple and adjustable attachment of the Raspberry Pi v2 camera module. The following design information includes all required for procuring and/or making the parts for the PiRamid and its assembly for use.

Design part name (see Fig. 2. for part positioning)	File type	File name and Location Repository location: https://zenodo.org/record/7090151Repository permanent location (Digital Object Identifier) : https://doi.org/10.5281/zenodo.7090151
PiRamid Cap **(1)**	STL	PiRamidCap.stl https://zenodo.org/record/7090151/files/PiRamidCap.stl
PiRamid Middle (with Pi attachment) **(2)**	STL	PiRamidMiddle.stl https://zenodo.org/record/7090151/files/PiRamidMiddle.stl
PiRamid Bottom **(3)**	STL	PiRamidBottom.stl https://zenodo.org/record/7090151/files/PiRamidBottom.stl
PiRamid three-part imaging device	OpenSCAD	PiRamid3Part.scad https://zenodo.org/record/7090151/files/PiRamid3Part.scadNote : this design file contains all three components in the stacked pyramid; each component needs to be rendered to view or edit and should be rendered individually to output single pieces for 3D printing.
Camera module mount **(4)**	STL	CameraModuleMount.stl https://zenodo.org/record/7090151/files/CameraModuleMount.stl
PiRamid imaging stage **(5)**	STL	PyramidImagingStage.stl https://zenodo.org/record/7090151/files/PyramidImagingStage.stl
PiRamid imaging stage **(5)**	OpenSCAD	PyramidImagingStage.scad https://zenodo.org/record/7090151/files/PyramidImagingStage.scad
PiRamid Python Script	Text	PiRamidPythonScript.txt https://zenodo.org/record/7090151/files/PiRamidPythonScript.txt
White semi-transparent plastic sheet to diffuse light, such as White Opal 3 mm Acrylic	No design file- a simple rectangle	100 × 95 mm rectangle – can be laser cut, cut by hand or machined depending on local supply and machining options.

Design files are also available within a public GitLab repository: https://gitlab.com/mattlong29/PiRamid.

These components of the device were all designed in the open-source OpenSCAD computer-aided design package (https://openscad.org/), and the STL files exported from OpenSCAD were then sliced using PrusaSlicer 2.3.0 software and transferred via SD card to a 3D printer, to print the components using default printer parameters. We used the following printer and configuration options:

- 3D printer: Prusa I3 MK3
- Black PLA (Polylactic Acid): 1.75 mm
- Printing conditions 0.2 mm layer height, infill 20 %, and other conditions as recommended by the slicer software.
- No support
- Brim may be required to improve bed adhesion

We have successfully printed PiRamid parts on a Creality Ender 3 with a variety of PLA colours from different suppliers. Most coloured filament did affect colour of light within the box and we therefore advise using black (or white) PLA.

Bill of materials

Component	Qty/ unit	Cost per unit (GBP; price in November 2021)	Source of material
Raspberry Pi 3B+ (Note: other models of Raspberry Pi can be used, depending on the application)	1	28.25	RS Components; https://uk.rs-online.com/web/p/raspberry-pi/1373331
Raspberry Pi, v2 Camera Module, CSI-2 with 3280 × 2464 pixels resolution	1	20.25	RS Components; https://uk.rs-online.com/web/p/raspberry-pi-cameras/9132664/
Raspberry Pi Power Supply, Micro USB Type B with UK Plug Type 1.5 m	1	6.45	RS Components; https://uk.rs-online.com/web/p/raspberry-pi-power-supplies/1770225/
SanDisk Micro SD Card 32 GB	1	6.08	RS Components; https://uk.rs-online.com/web/p/micro-sd-cards/1231040/
DesignSpark ABS Case for use with Raspberry Pi 3B	1	4.62	RS Components; https://uk.rs-online.com/web/p/raspberry-pi-cases/1677047/
Raspberry Pi Camera Ribbon Cable – 300 mm - CTLCAMCABLEASSY-300 mm	1	1.19	CPC; https://cpc.farnell.com/pro-signal/ctlcamcableassy-300mm/cable-for-pi-camera-300mm-formed/dp/SC13282?st=raspberry%20pi%20ribbon%20cables
LED backlight panel 86 × 45 mm (manufacturer part number KWB-R8445W/1W)	2	2.98	Cool Components; https://coolcomponents.co.uk/products/white-led-backlight-module-large-45mm-x-86mm
6 in Breadboard Jumper Wires female-to-female (pack of 5)	1 pack	3.92	RS Components; https://uk.rs-online.com/web/p/breadboard-jumper-wires/1947654
RS PRO Single Core Control Cable, Black, 1.5 mm2 CSAm 1000 V, 30/0.25 mm Core Strands, 25 m (Optional if LED modules are wired permanently by soldering)	1	11.67	RS Components; https://uk.rs-online.com/web/p/hook-up-wire/8114429
RS PRO 1.75 mm PLA 3D Printer Filament, 250 g, Black (Black PLA excludes external light most effectively, but we found different brands or colours of PLA printed successfully.)	1	9.99	RS Components; https://uk.rs-online.com/web/p/3d-printing-materials/8320406
Diffuser sheet of opal white semi-transparent acrylic. Product name "3mm Heavy Opaque Acrylic" option "Opal" 100 mm × 95 mm rectangle	1	1.18	Can be cut to size or ordered e.g. from "Cut my plastic"; https://www.cutmyplastic.co.uk/acrylic-sheet/heavy-opaque/3mm/non-recycled/L100-W95/
Optional components			
3.5″ IPS Touch Screen for Raspberry Pi (GPIO/SPI)	1	24.00	The Pi Hut (Waveshare); https://thepihut.com/products/spi-3–5-320x480-ips-touch-screen-gpio
Universal Power Supply or USB power bank with pass-through function GM322 Mini UPS 7800MAH 12 V 2A – KTC5336FBA, (15.4 × 13.6 × 4.6 cm; 340 Grams) Any suitable power bank or battery could be used, but it must output 5 V at 2A via USB to power Raspberry Pi, and must be possible to plug into the mains charger without interrupting the output	1	26.99	Amazon https://www.amazon.co.uk/Docooler-Protection-Charger-Portable-Applications-White/dp/B07BF4SR6S
Elesa 69,811 Black Multiple Lobes Clamping Knob	1	1.56	RS Components; https://uk.rs-online.com/web/c/engineering-materials-industrial-hardware/knobs-levers-handles/clamping-knobs/

(*continued on next page*)

(*continued*)

Component	Qty/ unit	Cost per unit (GBP; price in November 2021)	Source of material
Epi-illumination Version			
LISIPAROI White LED Camera Light for Raspberry Pi	1	13.84	Farnell; https://uk.farnell.com/cyntech/lisiparoiwht-01/lisiparoi-white-led-camera-light/dp/2840710

The total cost for this PiRamid, including the 3D printer material PLA is ∼£120, without the 3.5-inch touchscreen or UPS. The performance of this product will not differ with the addition of the touchscreen, but it improves usability by allowing experiments to be started and progress checked without a remote desktop via a network connection. Network connections can be more difficult to access in a laboratory environment, for example, Wi-Fi radio signals only poorly penetrate metal incubators. The device can also be powered with a UPS or USB power bank for increased portability with an additional cost of £26.99. The PiRamid with a touchscreen can have a cost of ∼£145 with the additional inclusion of a UPS rising to ∼£180.

Build Instructions

The following steps provide a step-by-step guide for building and assembling the PiRamid. Please see Fig. 2. for a diagram of the assembly.

3D printed parts assembly and Pi attachment:

1. Slice the STL files downloaded from the repository using the preferred slicer programme for your 3D printer. Transfer files using an SD card to the 3D printer and allow to print using chosen colour PLA (black PLA is suggested to allow for better light control).
2. Install the later version of Raspberry Pi software Raspbian onto a formatted SD card for use on your Raspberry Pi (see Raspberry Pi website for instructions). With this, you will be required to understand some basic Python coding skills. If you wish to use the picamera Python library ensure the operating system selected supports this, either by installing Buster or an earlier release or by enabling legacy camera control as advised by the Raspberry Pi Foundation. The basic Python script for using the PiRamid is available within the file repository and can be edited as required.
3. Attach the 3D printed camera mount to the inside of the cap part of the PiRamid (as shown in Fig. 1 and Fig. 2.) using four M1 screws and nuts. Attach the camera module to the camera mount using two M1 screws. At this point, the camera module can also be attached to the mount and the Raspberry Pi using either the stock ribbon cable or the ribbon cable purchased in the bill of materials (different lengths for personal preference). There is a slit in the cap of the PiRamid to thread the ribbon cable. Please follow the manufacturer's instructions (Raspberry Pi instructions) for camera attachment and enabling the hardware in operating system options.
4. Attach the bottom half of the Raspberry Pi case using screws to the two screw holes on the side of the middle section of the PiRamid. Alternative cases can be used according to preference, or a case can be 3D printed using various open-source designs; we recommend the use of a case to enclose the Raspberry Pi to reduce the risk of short-circuiting electronics or exposing it to laboratory spillages.
5. The imaging stage requires a 100 mm × 95 mm rectangular piece of white diffuser plastic sheet, such as 3 mm opal acrylic. This should be purchased or cut using a laser cutter and placed into the rectangular space in the centre of the imaging stage piece.
6. Optionally, using a drill, make a 5 mm diameter hole in the top of the imaging stage for attachment of the lobe clamping knob. This will act as a handle to make it easier to remove the imaging stage when required for example to change samples (see Fig. 2. for placement).
7. Slot the imaging stage over the top of the light panel housing and stack the 3 interlocking PiRamid sections together.
8. Optionally, the UPS can be attached to the Raspberry Pi via a suitable USB cable, or other Raspberry Pi power supply systems are available.
9. Optionally, for the epi-illumination version, follow instructions for wiring the LISIPAROI White LED camera light for Raspberry Pi. The PiRamid Bottom (3) can be printed and used as a base. For this option, we recommend printing in white PLA to have a white background.

Electrical wiring

1. Slot the LED light panels into the housing in the bottom section of the PiRamid. Make sure the connection wires are sticking out of the two holes printed at one end of the housing. Take care with the LED wires as they can break if bent repeatedly.
2. Using jumper wires, wire the positive (long wire) of each LED panel to a 33-ohm resistor. Users can attach these using jumper wires for speed and convenience, or permanent use by soldering using the appropriate wire. A 33-ohm resistor was found to deliver suitable brightness for the LED backlight panels used here, however, different resistors could be chosen depending on the voltage drop and target current if different LED modules are used. The switched GPIO pins deliver 3.3v and care should be taken to ensure the LEDs and series resistors do not draw more than the specified 16 mA maximum current from these pins.
3. Using jumper wires again, attach the ends of each resistor to one of the GPIO switching pins on the Raspberry Pi board. Make sure to note the number of the switching pin as this will be reflected later in the Python script. The numbers of the pins used in the current example Python script are GPIO 5 and 17. If a touchscreen is to be attached using a GPIO connection, make sure different pins are used than those used to power and communicate with the touchscreen. This can be edited on the Python script depending on which pins you use.
4. Using similar jumper wires, you can attach the two negative wires of the LED panels to the Ground pin on the Raspberry Pi computer GPIO.
5. When switching LED on or off using the Python script, you independently switch the two LED panels by controlling the different pins you decide to wire these to. Although both LED panels could be powered by a single pin, care should be taken to avoid drawing more than the maximum 16 mA specified for each pin; an advantage of using two independent pins for the two LEDs is that each one can draw more current to be brighter.
6. Optionally, the 3.5-inch touchscreen can be attached directly to the Raspberry Pi. To do so, the positive wires for the LED panels must be positioned within the top six GPIO pins. This is because the screen will slot directly onto the remaining GPIO pins.

Operation Instructions

This PiRamid imaging device can be used to image a range of life science laboratory experiments from monitoring crystal growth, food and nutrition research and analytical microbiology. The device can be allowed to run for seconds up to days with image intervals as low as fractions of seconds for time-lapse imaging. We have found it possible to record high-resolution images up to around 4 per second, even at the maximum image resolution; faster frame rates are possible with lower image resolution. User instruction is as follows:

1. Carry the device to the desired location for imaging, be it a walk-in incubator, smaller incubator, or benchtop.
2. Plug the Raspberry Pi controlling the device either into the mains power with the appropriate power supply or into the UPS and allow Raspberry Pi to boot.
3. The device will run with a fully charged UPS, however, can be powered by the mains through the UPS and moved/-transferred by removing from mains power and running solely on the UPS.
4. Load either the Raspberry Pi command line or Python integrated development software (IDE) such as Thonny. Here, load the desired Python script that will run your imaging. The basic Python script for running the device can be found in the repository (labelled as PiRamidPythonScript.txt). Instructions on how to edit the Python script to take the desired number of images with intervals can be found embedded within the Python script itself. The commands are described as image_count and wait_time.
5. Edit the Python script to the desired length of time between images, and the required number of images to set for a whole experiment. For example, this can be an image taken every 15 mins for 24 h, or simply an image every 30 s for 10 mins.
6. Remove the imaging stage from inside the device to add samples, making sure any samples or areas of the sample to be imaged remain within the diffuser light window on the imaging stage to illuminate.
7. Return the imaging stage to the base unit of the PiRamid taking care to not spill any biological materials or liquid from your samples.
8. On the first operation, it is important to carefully focus the v2 camera lens, this benefits from the remote desktop operation of the Raspberry Pi or connecting a large HDMI display, to see images in full definition to ensure sharp images. To focus the v2 camera module, a small plastic ring device (typically supplied with the camera module) is used to rotate the lens; unscrewing the lens is required to focus closer than when supplied, where these modules are typically supplied focussed near infinity to image objects at a significant distance. Depending on the height of the holder in the bottom tray, and the thickness of the sample, small focus adjustments might be needed for different tests.
9. The device can either be left on a shelf in a walk-in incubator (either plugged into mains or via UPS) or can be placed in a bench-top incubator when used for temperature-controlled experiments. Similarly, the device can be placed in any temperature-controlled room.

10. Make sure the cap containing the camera module is placed carefully on top of the device and that the device is running the Python script. When the experiment has started, check that images are being taken of the correct area and with suitable image quality by looking in the folder destination set within the Python script. Images are saved as soon as they are taken, allowing remote desktop or FTP to check the initial images before leaving the experiment to run e.g., overnight.
11. All 3D printed parts can be completely cleaned after use with detergent or antimicrobial such as 70 % alcohol to ensure sterility before and after use. Damaged or contaminated parts can be reprinted and replaced easily.

Validation and characterisation

To demonstrate the application of this device we compared a wide range of different experimental methods that might benefit from controlled, programmable time-resolved imaging. We explored different microbiology applications including bacterial growth assays in microfluidic devices and conventional agar colony culture using dip slides and Petri dishes, soft agar bacterial motility testing. Finally, we imaged crystal growth in a petri dish and also degradation of salad leaves, illustrating more diverse applications of time-lapse laboratory imaging. In these demonstrations we operated the PiRamid from battery or mains powered, and with minimal modification we achieved epi-illumination simply by adding an off-the-shelf LED ring for the Raspberry Pi camera, although most applications were brightfield illuminated with a white LED backlight.

We explored whether colourimetric analysis could be recorded successfully in microfluidic assays of resazurin growth indicators to produce growth curves, using a quality control reference strain of *E. coli* (ATCC 25922). To do this, MCF was coated internally with a hydrophilic layer of 5 mg/mL polyvinyl alcohol (PVOH) solution in water and incubated at room temperature for >2 h [28]. The coated MCF was then washed with 0.5 % Tween solution to remove any residual PVOH and left to dry on a vacuum manifold for 20 min. Individual MCF strips measured 17 mm in length with an internal capillary volume of 1 μL. In this case, six strips were clipped into 3D printed reusable 'combs', allowing the strips to be handled and dipped in a row of a 96-well plate and draw up the samples within the wells [14]. 3D printed end covers were filled with Dow Corning vacuum grease to prevent sample evaporation and slid over the ends of the MCF strips.

Reference strain *E. coli* 25922 was cultured on LB agar overnight at 37 °C. Colonies were scraped from the plate and suspended in Mueller-Hinton (MH) broth. Bacteria were grown for several hours until turbid and the final bacteria inoculum was diluted according to a 0.5 McFarland standard. MH broth was spiked with a bacterial suspension grown overnight at 37 °C in a 96-well plate with resazurin by 5-fold serial dilution using a micropipette. The bacterial concentrations ranged from 10^6 > to 10^3. The dipping of test strips into each well allowed for the sample to be taken up by capillary action. Samples were incubated overnight at 37 °C. Growth kinetics were able to be recorded using timelapse imaging from the PiRamid; resazurin conversion to the resorufin (dark blue to pink colour changes) was recorded every 15 min over a 20 hr incubation period. Images were analysed using MATLAB scripts to provide absorbance values which can be plotted against time. With resazurin dye reduction colour change from dark blue to pink, we see a change in absorbance values, allowing the plotting of a growth curve (Fig. 4.). Generation time can be calculated and used to estimate starting cell density in a sample (CFU/mL).

Alongside microfluidic bacterial growth assays, we show that we can image bacterial growth on agar and can identify individual colonies on 3D printed customised multi-sample dip-slides [12] and 50 mm diameter Petri dishes. For the custom dip slides, agar at 0.8 % in LB broth was autoclaved and, once cooled to 50℃, supplemented with a final concentration of

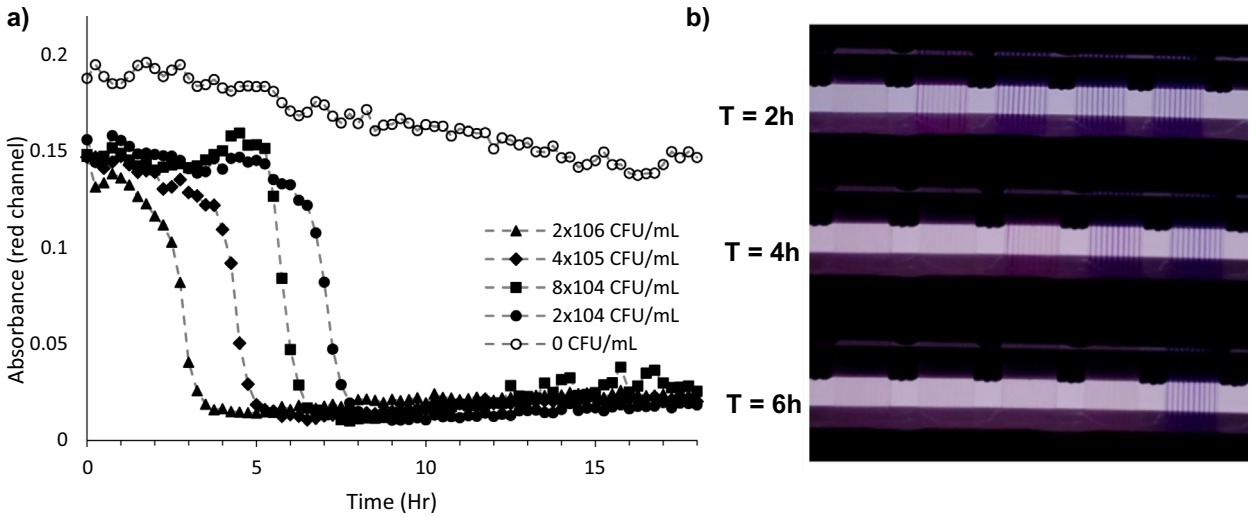

Fig. 4. Microfluidic colourimetric growth kinetics measurement of *E. coli* using resazurin dye, from time-lapse images taken in PiRamid (a) Growth curves for *E. coli ATCC 25922* in Mueller-Hinton broth were plotted for the indicated starting bacteria concentration (CFU/mL) in mirocapillary test strips. (b) PiRamid mages of MCF strips held in a 3D printed comb and containing 5-fold serial dilutions of E.coli ATCC 25922 grown in MH broth and resazurin dye. Four serial 5-fold dilutions of cell densities ranging from $2x10^6$ – $4x10^3$ CFU/mL from left to right; the final strip contains no bacteria and no growth was detected.

0.1 mg/mL Triphenyl tetrazolium chloride (TTC, Sigma Aldrich) which produces a dark stain following microbial growth, making colonies strongly coloured for brightfield imaging. Agar was added by micropipette into the wells of dip slides. Bacteria were grown overnight in MH broth and normalised to 0.5 McFarland standard and diluted to 1 in 10,000. Each dip slide was dipped into a 50 ml falcon tube containing diluted bacteria inoculum and then placed inside the PiRamid which was incubated overnight at 37C with images collected at regular intervals. Custom transparent covers for dip slides were used to prevent evaporation. The images were taken every 15 min over 20 h using the PiRamid allowing for the creation of a time-lapse video of bacterial growth with colonies stained by the TTC dye, used for the enumeration of bacterial colonies on solid culture media (Fig. 5). Time-lapse imaging allows clear visualisation of organism growth kinetics, with the potential to compare and quantify the growth of different species and in different growing conditions.

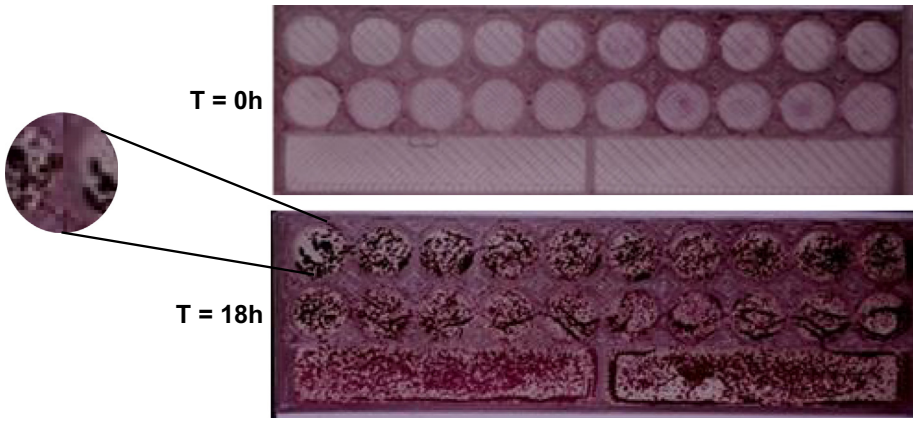

Fig. 5. Time lapse imaging of custom 3D printed agar dip slides (T. T. Diep et al., 2022). These 3D printed dip-slide devices have the potential for bacterial enumeration and identification for rapid testing in the field. Imaging using the PiRamid permits time-resolved imaging of microbial growth.

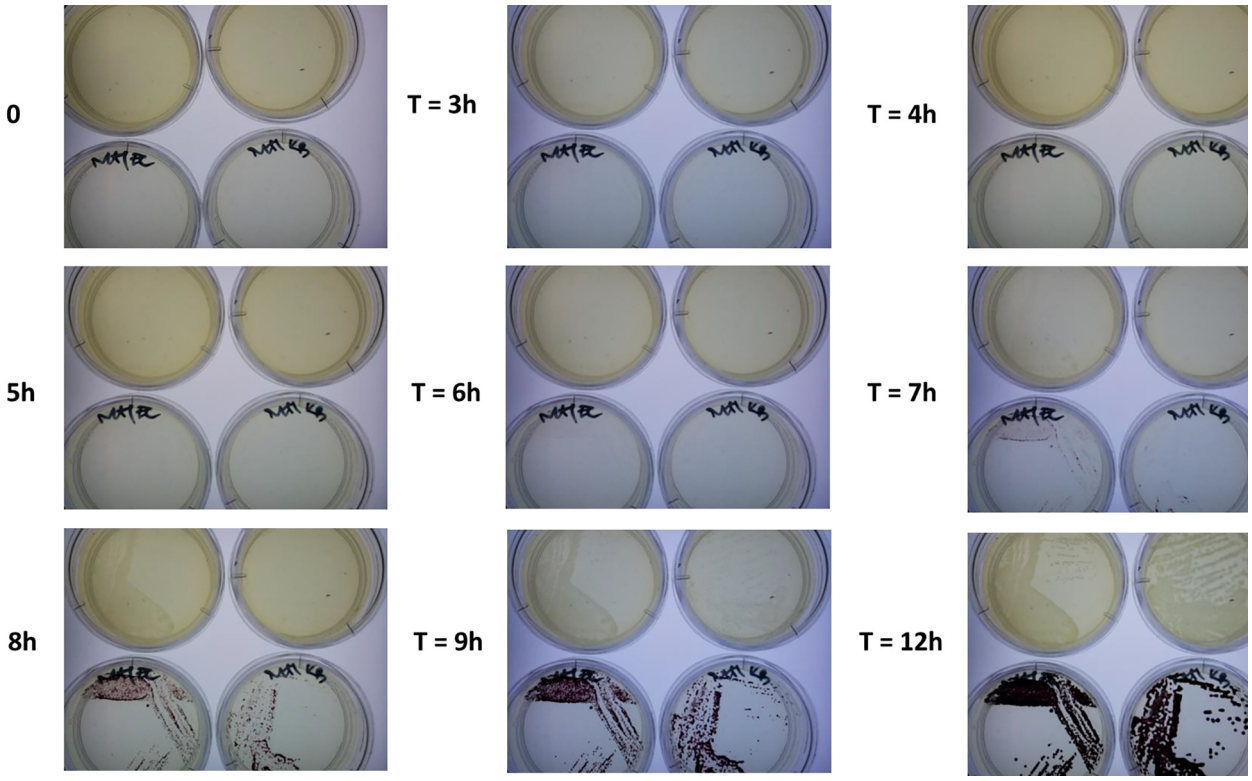

Fig. 6. Time lapse imaging of bacteria growth in petri dish. Images of *E.coli* (left) and *Klebsiella* (right) streaked on agar (top plates) and onto MH agar containing 0.5 mg/mL TTC dye to provide dark staining of microbial growth.

To observe traditional solid media culture, *E.coli* and *Klebsiella* colonies suspended in MH broth (normalised to 0.5 McFarland standard) were streaked onto 50 mm diameter Petri dishes. Images were taken every 10 min over 20 h using the PiRamid delivering a time-lapse video of bacterial growth either with unstained colonies, or the farm more clearly visible colonies when stained by TTC dye, often added for the enumeration of bacterial colonies on solid culture media (Fig. 6).

The final microbiological application we describe is motility testing. While conventional motility assays are interpreted at an endpoint, kinetic analysis might provide more detailed analysis of bacterial motility. For example, the effect of stimuli or inhibitors on growth and motility, such as inhibition, delays, or increased movement. Bacterial suspensions were stabbed into LB agar (0.4 %) containing TTC dye at a final concentration of 0.5 mg/ml in sterile polystyrene strip wells. TTC dye

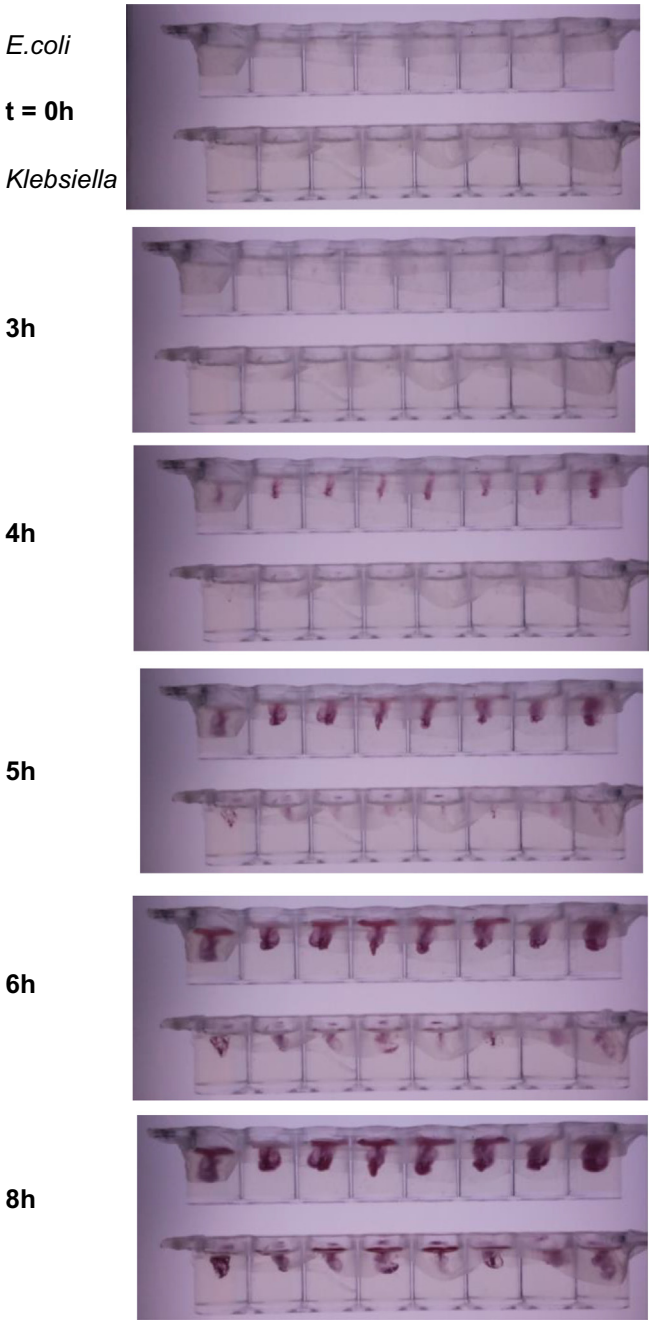

Fig. 7. Time lapse imaging of bacteria growth motility assay. Two species of bacteria (*E. coli* and *Klebsiella*) were stabbed into soft LB agar containing TTC dye at a final concentration of 0.5 mg/ml, which produces vivid dark staining where bacteria are present. This distinguishes motile non-motile bacteria enabling phenotypic identification. Here, *Klebsiella* is noted to be non-motile as only the immediate area inoculated with bacteria become stained; in contrast, a cloud of motile bacteria emerge from the stab site for *E. coli*.

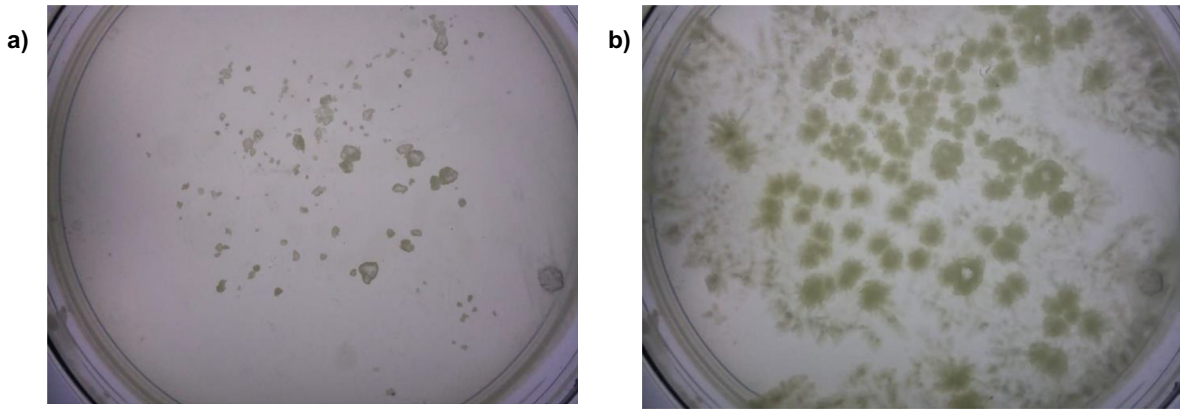

Fig. 8. Start and finish images of methyl anthranilate crystal formation Image of methyl anthranilate in water at start (a) with crystal growth appearing after 5 mins incubation at room temperature (b). Details of crystal growth can be seen with associated time lapse video available in the project repository https://zenodo.org/record/7090151.

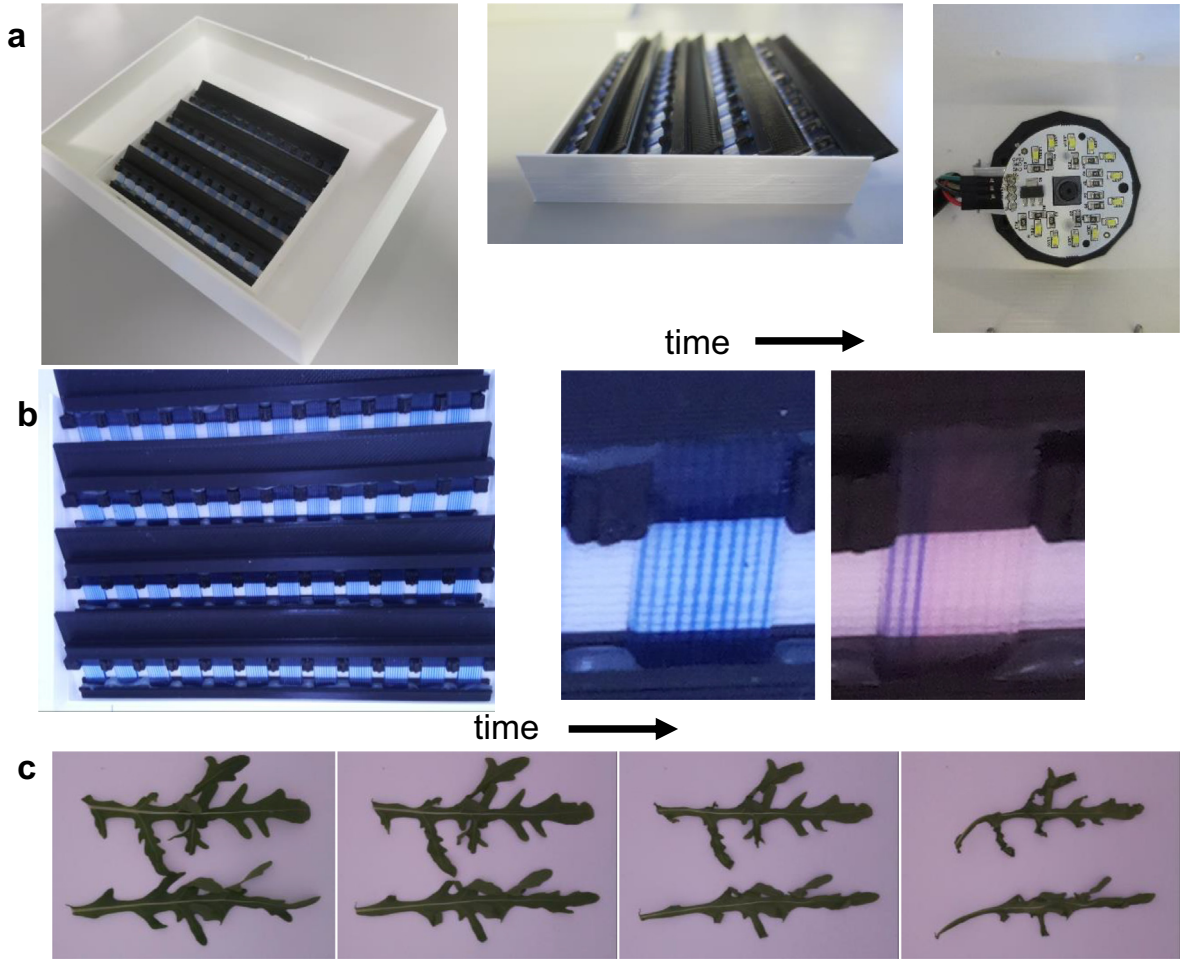

Fig. 9. Simple rapid customisation illustrated by adaptation for epi-illumination using LED light ring added to v2 camera module. a) Images of simple 3D printed base tray for 48 microfluidic test strips and LED ring light attachment inside top of PiRamid. b) Example image of 48x 10-capillary microfluidic bacterial growth strips (left), each containing a panel of antibiotics. Colorimetric growth by change over time from blue resazurin to pink-white can clearly be detected as well with top-light on white PLA 3D printed base tray (right) as with brightfield using LED panels under diffuser (Fig. 4). c) Toplight illumination clearly records deterioration of salad leaves over time, illustrating a broader range of samples can be imaged using distinct lighting configuration.

was added to stain bacteria improving image contrast. The images obtained clearly demonstrate the different behaviour of motile vs non-motile bacteria (Fig. 7). As expected, *Klebsiella* is non-motile with staining limited to the stab site; in contrast a cloud of *E. coli* moves away from the stab, indicating the ability to swim through the soft agar.

To explore alternative applications, we asked if the system could record dynamics of crystal growth, using methyl anthranilate. This is an ester of anthranilic acid and is widely used as a flavouring and scenting agent in food and drink products. Methyl anthranilate was chosen as a simple organic compound (melting point 24 ℃) that has been found to grow crystals readily (<5 mins). Other crystal systems take significantly longer to form crystals, which would benefit from automated viewing. Solid methyl anthranilate was heated at 37 ℃ in an incubator in a laboratory petri dish of water, followed by cooling to 4 ℃. When placed in the PiRamid at room temperature, a small amount of solid methyl anthranilate was added to seed crystallisation. The resulting crystal growth was imaged every-five seconds for up to five minutes in total and a time-lapse video was produced from the resulting images, with crystal growth clearly visible by comparing endpoint with start image (Fig. 8). This setup could be combined with real-time image analysis software to notify scientists when crystals have grown large enough for analysis. This could be a useful automation tool for panels of crystallisation samples without in-person supervision.

Finally, to demonstrate the flexibility of the system, we added an off-the-shelf LED ring module to the v2 camera, and replaced the base tray with a deeper tray, printed in white PLA to provide epi-illumination (Fig. 9). We tested this top-light configuration with a panel of 48 microfluidic microbiology test strips (480 × 1 µL samples) and with two salad leaves. Time-resolved imaging of the microcapillaries allowed bacterial growth kinetics to be detected, with similar results to the brightfield setup with backlight. Similarly, degradation of the salad leaves was clearly visible over time, with great potential to quantify shelf-life properties of food products. The rocket leaves were imaged every hour for 22 h at room temperature and the wilting of the leaves can be clearly observed.

In conclusion, PiRamid is useful as a compact, small experiment imaging device, and is limited by its size and therefore the number of samples capable of being imaged. However, for larger studies needing more samples its affordability and easy construction would allow the user to purchase the materials needed and construct with ease multiple devices that could be used in parallel. For example, for bacteriology and microfluidics, you could run experiments with greater numbers of isolates by using a cluster of multiple PiRamids.

For all above experiments, time-lapse videos can be located in the Zenodo repository, as indicated in Table 1.

Table 1

Figure	File name within published repository: https://zenodo.org/record/7090151 https://doi.org/10.5281/zenodo.7090151
Figure 5. Time lapse imaging of custom 3D printed agar dip slides.	**E.coliGrowthDipSlide**
Figure 6. Time lapse imaging of bacteria growth in petri dish.	**E.coli + KlebPetriDish**
Figure 7. Time lapse imaging of bacteria growth motility assay.	**E.coli + KlebMotility**
Figure 8. Time lapse imaging of methyl anthranilate crystal formation.	**MethylAnthranaliteCrystalFormation**

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] Y. Fan, J. Li, Y. Guo, L. Xie, G. Zhang, Digital image colorimetry on smartphone for chemical analysis: a review, Measurement 171 (2021), https://doi.org/10.1016/J.MEASUREMENT.2020.108829 108829.

[2] L.F. Capitán-Vallvey, N. López-Ruiz, A. Martínez-Olmos, M.M. Erenas, A.J. Palma, Recent developments in computer vision-based analytical chemistry: a tutorial review, Anal. Chim. Acta 899 (2015) 23–56, https://doi.org/10.1016/J.ACA.2015.10.009.

[3] M.I. Latypov, J.C. Stinville, J.R. Mayeur, J.M. Hestroffer, T.M. Pollock, I.J. Beyerlein, Insight into microstructure-sensitive elastic strain concentrations from integrated computational modeling and digital image correlation, Scr. Mater. 192 (2021) 78–82, https://doi.org/10.1016/J.SCRIPTAMAT.2020.10.001.
[4] A.A. Mohamed, A.A. Shalaby, A.M. Salem, The Yxy colour space parameters as novel signalling tools for digital imaging sensors in the analytical laboratory, RSC Adv. 8 (19) (2018) 10673–10679, https://doi.org/10.1039/C8RA00209F.
[5] M. Ferone, A. Gowen, S. Fanning, A.G.M. Scannell, Microbial detection and identification methods: Bench top assays to omics approaches, Compr. Rev. Food Sci. Food Saf. 19 (6) (2020) 3106–3129, https://doi.org/10.1111/1541-4337.12618.
[6] W. Ouyang, R.W. Bowman, H. Wang, K.E. Bumke, J.T. Collins, O. Spjuth, J. Carreras-Puigvert, B. Diederich, An open-source modular framework for automated pipetting and imaging applications, Adv. Biol. 6 (4) (2022) 2101063.
[7] D. Deb, S. Hariharan, U.M. Rao, C.H. Ryu, Automatic detection and analysis of discontinuity geometry of rock mass from digital images, Comput. Geosci. 34 (2) (2008) 115–126, https://doi.org/10.1016/J.CAGEO.2007.03.007.
[8] X. Huang, D. Xu, J. Chen, J. Liu, Y. Li, J. Song, X. Ma, J. Guo, Smartphone-based analytical biosensors, Analyst 143 (22) (2018) 5339–5351.
[9] S.İ. Dönmez, S.H. Needs, H.M.I. Osborn, A.D. Edwards, Label-free smartphone quantitation of bacteria by darkfield imaging of light scattering in fluoropolymer micro capillary film allows portable detection of bacteriophage lysis, Sens. Actuators B 323 (2020), https://doi.org/10.1016/j.snb.2020.128645 128645.
[10] M.P. Walzik, V. Vollmar, T. Lachnit, H. Dietz, S. Haug, H. Bachmann, M. Fath, D. Aschenbrenner, S. Abolpour Mofrad, O. Friedrich, D.F. Gilbert, A portable low-cost long-term live-cell imaging platform for biomedical research and education, Biosens. Bioelectron. 64 (2015) 639–649.
[11] B. I. Morris et al., 'PiSpy: An Affordable, Accessible, and Flexible Imaging Platform for the Automated Observation of Organismal Biology and Behavior', bioRxiv, p. 2022.03.21.485129, Mar. 2022, doi: 10.1101/2022.03.21.485129..
[12] T.T. Diep, P.P. Ray, A.D. Edwards, Methods for rapid prototyping novel labware: using CAD and desktop 3D printing in the microbiology laboratory, Lett. Appl. Microbiol. 74 (2) (2022) 247–257, https://doi.org/10.1111/lam.13615.
[13] S. H. Needs, Z. Rafaque, W. Imtiaz, P. Ray, S. Andrews, D. Edwards, 'High-throughput, multiplex microfluidic test strip for the determination of antibiotic susceptibility in uropathogenic E. coli 2 with smartphone detection', bioRxiv, p. 2021.05.28.446184, May 2021, doi: 10.1101/2021.05.28.446184..
[14] N.M. Reis, J. Pivetal, A.L. Loo-Zazueta, J.M.S. Barros, A.D. Edwards, Lab on a stick: multi-analyte cellular assays in a microfluidic dipstick, Lab Chip 16 (15) (2016) 2891–2899, https://doi.org/10.1039/c6lc00332j.
[15] S.Y. Teh, R. Lin, L.H. Hung, A.P. Lee, Droplet microfluidics, Lab Chip 8 (2) (2008) 198–220, https://doi.org/10.1039/B715524G.
[16] S. Sohrabi, N. Kassir, M. Keshavarz Moraveji, 'Droplet microfluidics: fundamentals and its advanced applications', *RSC*, Advances 10 (46) (2020) 27560–27574, https://doi.org/10.1039/D0RA04566G.
[17] R. Albernaz-Gonçalves, G. Olmos, and M. J. Hötzel, 'Exploring Farmers' Reasons for Antibiotic Use and Misuse in Pig Farms in Brazil', Antibiotics 2021, Vol. 10, Page 331, vol. 10, no. 3, p. 331, Mar. 2021, doi: 10.3390/ANTIBIOTICS10030331..
[18] V. Kasimanickam, M. Kasimanickam, and R. Kasimanickam, 'Antibiotics Use in Food Animal Production: Escalation of Antimicrobial Resistance: Where Are We Now in Combating AMR?', Medical Sciences 2021, Vol. 9, Page 14, vol. 9, no. 1, p. 14, Feb. 2021, doi: 10.3390/MEDSCI9010014..
[19] S.H. Needs, T.T. Diep, S.P. Bull, A. Lindley-Decaire, P. Ray, A.D. Edwards, I. Kusters, Exploiting open source 3D printer architecture for laboratory robotics to automate high-throughput time-lapse imaging for analytical microbiology, PLoS One 14 (11) (2019), https://doi.org/10.1371/journal.pone.0224878.
[20] J.M. Pearce, Impacts of open source hardware in science and engineering, Bridge 47 (3) (2017).
[21] S. Steffens, L. Nüßer, T.-B. Seiler, N. Ruchter, M. Schumann, R. Döring, C. Cofalla, A. Ostfeld, E. Salomons, H. Schüttrumpf, H. Hollert, M. Brinkmann, R.L. Tanguay, A versatile and low-cost open source pipetting robot for automation of toxicological and ecotoxicological bioassays, PLoS One 12 (6) (2017), https://doi.org/10.1371/JOURNAL.PONE.0179636.
[22] Q. Lu, G. Liu, C. Xiao, C. Hu, S. Zhang, R.X. Xu, K. Chu, Q. Xu, Z.J. Smith, K.C. Maitland, A modular, open-source, slide-scanning microscope for diagnostic applications in resource-constrained settings, PLoS One 13 (3) (2018), https://doi.org/10.1371/JOURNAL.PONE.0194063.
[23] BIOLOG, 'Omnilog – Bacteria, Yeast and Fungi Identification'. 2017..
[24] T.T. Diep, S. Bizley, P.P. Ray, A.D. Edwards, MicroMI: A portable microbiological mobile incubator that uses inexpensive lithium power banks for field microbiology, HardwareX 10 (2021) e00242.
[25] A. Maia Chagas, L.L. Prieto-Godino, A.B. Arrenberg, 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 Biol. 15 (7) (2017) e2002702.
[26] J.P. Sharkey, D.C.W. Foo, A. Kabla, J.J. Baumberg, R.W. Bowman, A one-piece 3D printed flexure translation stage for open-source microscopy, Rev. Sci. Instrum. 87 (2) (2016), https://doi.org/10.1063/1.4941068 025104.
[27] R.Y. Neches, K.J. Flynn, L. Zaman, E. Tung, N. Pudlo, On the intrinsic sterility of 3D printing, PeerJ 12 (2016) 2016, https://doi.org/10.7717/PEERJ.2661.
[28] J. Pivetal, F.M. Pereira, A.I. Barbosa, A.P. Castanheira, N.M. Reis, A.D. Edwards, Covalent immobilisation of antibodies in Teflon-FEP microfluidic devices for the sensitive quantification of clinically relevant protein biomarkers, Analyst 142 (6) (2017) 959–968, https://doi.org/10.1039/C6AN02622B.

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