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Pi USB Cam: A simple and affordable DIY solution that enables high-quality, high-throughput video capture for behavioral neuroscience research

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ABSTRACT

Video recording is essential for behavioral neuroscience research, but the majority of available systems suffer from poor cost-to-functionality ratio. Commercial options frequently come at high financial cost that prohibits scalability and throughput, whereas DIY solutions often require significant expertise and time investment unaffordable to many researchers. To address this, we combined a low-cost Raspberry Pi microcomputer, DIY electronics peripheries, freely available open-source firmware, and custom 3D printed casings to create Pi USB Cam, a simple yet powerful and highly versatile video recording solution. Pi USB Cam is constructed using affordable and widely available components and requires no expertise to build and implement. The result is a system that functions as a plug-and-play USB camera that can be easily installed in various animal testing and housing sites and is readily compatible with popular behavioral and neural recording software. Here, we provide a comprehensive parts list and step-by-step instructions for users to build and implement their own Pi USB Cam system. In a series of benchmark comparisons, Pi USB Cam was able to capture ultra-wide fields of view of behaving rats given limited object distance and produced high image quality while maintaining consistent frame rates even under low- and no-light conditions relative to a standard, commercially available USB camera. Video recordings were easily scaled using free, open-source software. Altogether, Pi USB Cam presents an elegant yet simple solution for behavioral neuroscientists seeking an affordable and highly flexible system to enable quality video recordings.

SIGNIFICANCE STATEMENT

Video capture is increasingly necessary for neuroscience research where neural and behavioral data are synchronized to reveal correlative and causal relationships. This relies on a recording system that can capture quality videos without significant alterations to pre-existing experimental conditions (e.g., lighting, space, etc.), enables easy online and offline analysis by commonly used software, and offers high scalability to increase throughput. However, the high cost and poor flexibility of commercially available options leave the role of an ideal video recording system unfulfilled. Here, we present a DIY video recording solution that combines affordable electronics hardware and custom 3D printed components with sophisticated open-source software to make a simple, yet powerful USB camera that satisfies almost any recording need.

INTRODUCTION

Video recorded data is a crucial component of behavioral neuroscience research. While a number of commercially available recording solutions exist, few, if any, satisfy all or most of the needs of a typical laboratory. Many behavioral assays, particularly those intended for use with nocturnal species, are conducted under low- or no-light conditions to mimic the portion of the circadian cycle during which animals are the most active. Therefore, an ideal video recording system enables video acquisition under both ambient and infrared (IR) illumination. Moreover, spatial limitations imposed by testing apparatuses frequently require capture of a large field of view from a relatively short object distance. Although some commercially available options designed specifically for behavioral neuroscience research meet these criteria, they are often expensive and unique to a specific testing apparatus limiting scalability and flexibility of use. Given these limitations, researchers frequently repurpose commercial USB webcams for video acquisition because of their affordability and accessibility. However, webcams are rarely intended for recording under low- to no-light conditions, nor for capturing a large field of view under circumstances when subjects are at a relatively short distance from the camera.

DIY solutions that make use of low-cost, single board microcomputers such as Raspberry Pi have gained the attention of behavioral neuroscientists as laboratories attempt to address the shortcomings of existing commercially available solutions. For example, Singh et al. (2019) developed a powerful web-based interface for long duration, remote video recording and streaming. However, lack of active maintenance and development quickly rendered it obsolete as the current generation of Raspberry Pi board (4B) no longer supports the legacy OS (Raspbian Stretch) for which it was developed. Recently developed solutions that use the Raspberry Pi Camera in its most basic form to acquire video are less demanding in terms of debugging and updating (Saxena et al., 2018; Weber and Fisher, 2019; Clemensson et al., 2020; Centanni and Smith, 2021). However, these out-of-the-box options suffer from limited functionality, often lacking the capacity to live preview and record at the same time, or interface with closed-loop behavioral control systems. As a result, DIY solutions frequently require a degree of programming knowledge to properly configure and adapt the system to specific research needs thereby limiting broad application of such systems by novice users. Thus, drawbacks associated with both commercially available and DIY video capture solutions pose significant limitations to easy acquisition of video recorded behavioral data.

To address this gap, we designed a versatile, low-cost, DIY video recording solution that requires no specialized expertise or equipment. The Pi USB Cam is low-/no-light compatible and accepts a variety of wide-angle lenses. The system utilizes open-source, actively maintained software that enables true plug-and-play capability. Combined with custom 3D-printed components and freely available open-source video acquisition software, this system offers highly versatile implementation and scalability across numerous behavioral testing conditions. Here, we provide detailed step-by-step instructions for hardware and software setup and demonstrate its ease of use and superiority in terms of field of view and low-light recording over commercial counterparts.

MATERIALS AND METHODS

Build the camera

Pi USB Cam is comprised of a Raspberry Pi board (Raspberry Pi, Cambridge, Cambridge, UK) and a wide-angle day-night vision camera (Arducam Technology Co., Limited, Hong Kong, China). This camera comes equipped with a motorized IR-CUT filter that is automatically triggered based on ambient light intensity to accommodate for both bright-light and low-/no-light recording, as well as an out-of-the-box 170° [Diagonal FOV] x 140° [Horizontal FOV] fisheye lens. The camera uses a 5MP OV5647 sensor, which can support up to 30 frames per second (FPS) at 1080p when used with the suggested firmware. Additionally, the camera comes attached with two 850 nm infrared LEDs to facilitate image acquisition in low-/no-light conditions.

We selected the Raspberry Pi 4 Model B to run the camera, which was the latest release of the main product line at the time of publication. This model and the more affordable and compact Raspberry Pi Zero are readily supported by *Show-me webcam*, an open-source firmware that enables a Raspberry Pi connected camera to be booted as a simple USB camera. While both models are compatible as the base hardware for building a Pi USB Cam, we chose Pi 4B over Zero due to its added processing power, which is significantly more advantageous if the Pi board were to be repurposed for GUI applications. Of note, while the Raspberry Pi Zero 2 was recently released, it is not yet supported by *Show-me webcam*, although this is likely to change in near future. Legacy boards like Pi 3+ and earlier releases that are no longer widely accessible for purchase are not supported by *Show-me webcam*.

A complete list of store-bought components is provided in **Table 1** with a more comprehensive list including alternative options provided in **Extended Table 1-1**.

<u>Step-by-step instructions</u>

Software installation

1. Insert a clean micro-SD card with a minimum storage of 64MB into your computer **(Figure 1A)**.
2. Download the latest release of the *Show-me webcam* image file to your computer from the developer's GitHub page https://github.com/showmewebcam/showmewebcam **(Figure 1B)**. Make note to download the image file corresponding to the appropriate Raspberry Pi board model.
3. Download, install, and launch the official Raspberry Pi Imager from https://www.raspberrypi.com/software/ **(Figure 1C)**. Inside the imager's main interface, click on **CHOOSE OS** followed by **Use custom** to locate the image file you just downloaded **(Figures 1C1-2)**. Then, click on **CHOOSE STORAGE** and select the clean micro-SD card **(Figures 1C3-4)**. Lastly, click on **WRITE** followed by **YES** on the pop-up warning to flash the *Show-me webcam* image file into the micro-SD card **(Figures 1C5-6)**.

Hardware Assembly

1. Gather the essential hardware components shown in **Figure 2A** and appropriate housings if desired for the Pi board **(Table 1)** and camera **(Table 2)**.

   OPTIONAL: If you intend to use the camera for low-/no-light recording close to a reflective surface such as plexiglass, we recommend removing the IR LEDs that come attached to the camera module **(Figure 2B)** at this stage. Doing so will allow for flexible LED placement thereby enabling the user to avoid flaring artifacts in the captured image **(Figure 4A)**. For more information on how to use and position LEDs independently from the camera refer to "**IR LED Setup**" and see **Figure 4**.
2. To connect the camera module to the Pi board, first locate the camera ribbon cable ports on the camera module and the Pi board, as indicated in **Figure 2C1**. Gently lift the black plastic clip on the ports. Insert the ribbon cable terminal making sure that the silver leads on

the cable face the contacts inside the port. Push down the plastic clip to secure the ribbon cable in place **(Figure 2C2)**.

3. Insert the micro-SD card prepared in *Software Installation* into the micro-SD card slot on the back of the Pi board to finish the setup **(Figure 2D)**.
4. To power up and start using the Pi USB Cam, connect it to a host computer via its USB-C port using a USB cable **(Figure 2E1)**. The red built-in LED should stay lit to indicate that it is receiving adequate power supply **(Figure 2E2)**. The green built-in LED will fast blink three times after booting, which will take around 5 seconds, to indicate that the Pi USB Cam is ready for use. This LED will remain illuminated when the Pi USB Cam is in active use **(Figure 2E2)**.
5. You can optionally house the Pi USB Cam in our custom 3D-printed camera case for protection and use mount tools for easy installation in a variety of behavioral testing configurations **(Figures 2F, 9-11)**. STL files and print instructions can be found at our Thingiverse page: https://www.thingiverse.com/gloverlab/designs.

   NOTE: The STL files provided can be readily sliced by popular slicing software and printed with consumer-grade 3D printers or commercial pay-to-print service offered by universities and elsewhere. For an estimated cost of printing a full set of our camera and IR LED holders using either printing options, see **Extended Table 1-2**.

Adjusting camera settings

The Arducam day-night vision camera module that we suggest using here has an on-board photoresistor **(Figure 3A1)** that senses ambient light intensity. This allows for automatic control of the IR filter to enable IR sensitivity under low-/no-light conditions **(Figure 3B)** and improve color accuracy under bright light conditions **(Figure 3C)**. However, in scenarios where lighting conditions change dramatically within a single recording or approach the ambient light threshold, one might consider manually disabling the motorized IR filter to prevent automatic IR filter shuttering and keep it in either a permanent ON or OFF state throughout the entire recording session. Covering the photoresistor with non-translucent tape will prevent ambient light from reaching the sensor thereby enabling IR sensitivity by permanently turning the IR filter OFF **(Figure 3A1)**. Conversely, to manually enable IR correction, the IR filter can permanently be switched ON by unplugging the motorized IR filter connector from the back of the camera board **(Figure 3A2)**.

Camera software settings such as brightness and white balance can also be readily adjusted to suit specific recording needs, such as improving the image color when recording under low-light conditions **(Figure 3B).** Users can access camera setting parameters by interfacing the Pi USB Cam with a host computer. The following protocol (adapted from https://tutorial.cytron.io/2020/12/29/raspberry-pi-zero-usb-webcam/) describes how to access and adjust camera setting parameters on a Windows PC using an open-source software called PuTTY **(Figure 3G)**, which allows the PC to establish a serial connection with Pi USB Cam. The same can be accomplished on a Linux or Mac computer via command line (visit the official debugging guide for more: https://github.com/showmewebcam/showmewebcam).

Step-by-step instructions

1. To identify which USB serial communication (COM) port on your host PC the Pi USB Cam is using, open **Device Manager** and locate the COM port number of the camera under **Port (COM & LPT) (Figure 3E)**.

   NOTE: If you have more than one USB serial device connected to your computer, you can disconnect and then reconnect Pi USB Cam while monitoring hardware changes in **Device Manager** to confirm which COM port is used by which camera.

2. Download, install, and launch PuTTY from https://www.putty.org. To establish a serial connection with the Pi USB Cam, first make sure that **Session** is selected under **Category (Figure 3F1)**. Then set **Connection type** to **Serial (Figure 3F2)**, enter the

COM port number of the camera under **Serial line (Figure 3F3)**, and set **Speed** to **112800 (Figure 3F4)**. Click **Open** to log into the Pi USB Cam **(Figure 3F5)**.

3. Once logged in, type the following command `/usr/bin/camera-ctl` and press **Enter** to launch the **camera-ctl** interface and show all adjustable camera setting parameters **(Figure 3G)**. These can be adjusted during live preview to see how any changes affect image quality.

   OPTIONAL: We recommend setting **Video Bitrate** to **Maximum (25000000)**, and **Auto Exposure**, **White Balance**, and **ISO Sensitivity Auto** to **Manual (Figure 3G2)**, to improve the image coloring under low-light conditions **(Figure 3B)**. We also set **Brightness** to **53** for all low-light recordings used in this paper. For recording under bright-light conditions, consider reverting **White Balance** to the default **Auto** to improve color accuracy **(Figure 3C)**. However, we encourage experimenting with the setting as it likely varies with the recording condition and recording devices.
4. Once finished, press **S** to save the changes in the camera setting parameters, or revert back to default by pressing **D** (resets individual setting to default) or **R** (resets all settings to default). To terminate the serial connection, first press **Q** to quit the **camera-ctl** interface, and then close the PuTTY session window.
5. To adjust the focus on the camera lens, first loosen the screw that secures the lens firmly inside the M12 lens holder, as indicated in **Figure 3H**, then simply turn the lens clockwise or counterclockwise while monitoring the camera preview until the image becomes sharp.

IR LED setup

IR-sensitive cameras need to be coupled with adequate IR illumination to produce high-quality images in low-/no-light conditions. The wide-angle day-night vision camera from Arducam that we suggest using in this configuration comes with two 850nm IR LEDs attached and powered by the camera board **(Figure 2B)**. However, recording through reflective surfaces such as plexiglass across short object distances (e.g., operant box) caused undesirable flare artifacts in the field of view **(Figure 4A)**. To circumvent this, we removed the LED boards from the main camera board and powered them independently using a pair of 3v3 power and ground pins on the Raspberry Pi board **(Extended Figure 2-1)**. This allowed LED placement independent of the camera enabling even and high-quality illumination of the area of interest **(Figures 4B-C)**. This section describes the steps to wire two 850nm LEDs in parallel using custom-made jumper cables and power them with the Pi board itself **(Figures 4D-G)**.

NOTE: The 3v3 power pin of the Pi board can safely provide up to 500mA of current (according to: https://pinout.xyz/pinout/3v3_power). Exceeding that limit using high-power LEDs may cause a “brownout” of the Pi and potential safety concerns. Our testing indicated that the two IR LED boards that came with the Arducam day-night vision camera draw ≤ 200mA of current from the 3v3 pin when connected in parallel. For higher power LEDs, it is recommended to use either the 5v power pin, which provides a higher current draw of about 1.5A (according to: https://pinout.xyz/pinout/5v_power), or an external battery pack or power supply unit. For alternative LED options and related hardware, see **Extended Table 1-1**.

Step-by-step instructions

1. Following the step where the LED boards were taken off from the main camera board **(Figure 2B)**, cut three segments of 22-gauge Red-Black electrical wire **(Figure 4D)**: **(1)** a short ~3 cm wire to connect the two LED boards in parallel; **(2)** a long ~80 cm wire (adjust to specific installation requirements) for connecting the LED unit to the Pi board located some distance from the camera; **(3)** a short ~6 cm wire to connect wire **(2)** to a pair of 3v3 power and ground pins on the Pi board.

2. Split wire **(1)** down the middle into two separate red and black wires. Split one end of wires **(2)** and **(3)** by ~3 cm and the other end of each ~1 cm.
3. Crimp the appropriate electrical connectors to the stripped wires as shown in **Figure 4D**
   a. Install two non-insulated ring connectors on either end of wire **(1)**.
   b. Install two ring connectors on the end of wire **(2)** separated by ~3 cm and two male pin connectors housed in one 1x2 plastic housing on the other end.
   c. Install two female pin connectors each housed in 1x1 plastic housings on the end of wire **(3)** separated by ~3 cm and two female pin connectors housed in one 1x2 plastic housing on the other end.

   NOTE: For detailed instructions on how to crimp ring and pin type connectors, see **Extended Figures 4-1 & 4-2**.
4. Wire the LED boards in parallel as shown in **Figure 4E** and **Figure 4F1**.

   NOTE: Red wires are used for power (+) and black wires for ground (-). Make sure the polarities of all components align before completing the circuit.
   a. Secure the ring connectors on wires **(1)** and **(2)** to the LED boards using the screws that came with camera module.
   b. Mate the two female pin connectors on wire **(3)** to any pair of 3v3 power and ground pins on the Pi board 40-pin header (e.g., pins 1 and 6 as shown in **Figure 4E**).
   c. Connect wires **(2)** and **(3)** via their respective pin connectors to complete the circuit.

   Test functionality by powering up the Pi USB Cam and confirming that the LED unit is illuminated under low-/no-light conditions.
5. Install heat sinks on the back of the LED boards to prevent overheating **(Figure 4F)**. Optionally house the fully wired LED unit in the custom 3D-printed case and install the

fully assembled unit in any behavioral testing site using the 3D-printed mount tools **(Figure 4G)**.

Multi-camera video acquisition

Pi USB Cam affords a high degree of scalability that is often desired for high throughput experiments. Use of the freely available, open-source video acquisition software, OBS (Open Broadcaster Software) Studio enables preview of live video feeds on screen for real-time behavioral monitoring, live-stream video feeds over internet, and simultaneous video acquisition from multiple camera sources into separate local files for offline behavioral analysis. OBS studio is compatible with all three major operating systems; however, we recommend using a Windows PC as the host computer due to added functionalities including video configuration settings that are not available on other platforms. Scalability is limited only by the hardware of the host computer (e.g., number of USB controllers / ports, sufficient CPU, etc). We recommend using a Windows desktop that allows for add-on USB PCIe expansion cards to provide additional USB controllers and greater bandwidth for multi-camera recordings.

The following protocol describes how to set up OBS Studio on a Windows desktop for multi-Pi USB Cam video recordings. For streaming over network, adding audio recording, and many more applications, users can visit the OBS Wiki at https://obsproject.com/wiki/. Note that unless otherwise stated all videos and snapshots of videos included in Figures were acquired at 480p and 30 FPS using OBS Studio.

Step-by-step instructions

1. Connect one or more Pi USB Cams to a host Windows desktop that is optionally installed with USB PCIe expansion cards for added USB bandwidth **(Figure 5A)**.
2. To make sure that your multi-camera configuration will not exceed the USB bandwidth during recording, open **Device Manager**, click **View** followed by **Devices by**

connection, and confirm that no more than three Pi USB Cams are connected to each **Host Controller (Figure 5B)**.

3. Download, install, and launch the latest release of OBS Studio from: https://obsproject.com **(Figure 5C)**.

   NOTE: The default user interface can be customized under the **View** menu to hide unnecessary features such as the **Audio Mixer** and **Scene Transitions**.

4. Users can create one or more "profiles" that can save any set of customized recording settings. To do this, go to the **Profile** menu, and click **New (Figure 5D1)**. In the pop-up window, enter a name for the new profile (e.g., "480p30fps" to reflect the recording resolution and frame rate that will be updated in step **5**), uncheck the auto-configuration wizard, and click **OK** to finish creating the new profile **(Figure 5D2)**. Once a profile is selected, any changes in recording settings will be automatically saved under that profile.

   NOTE: One convenient way to use the "profile" feature is to create one for each experimental protocol that specifies its own recording needs and settings. Saved settings for each specific protocol can be quickly applied before each recording session by simply selecting the appropriate profile.

5. To customize recording settings, click **Settings** on the **Controls** panel **(Figure 5D3)** to open the setting window. **Figures 5D4-6** highlight several changes to the OBS Studio default settings that we recommend for behavior recordings. Click **OK** to save any changes to the recording settings and to close the pop-up window **(Figure 5D7)**.

6. In steps 6-9, we provide options to preview and record video from multiple video sources. OBS Studio refers to content being broadcast at any given time as a "scene" whereas a configuration of scenes and their respective video sources is referred to as a "scene collection". Users may want to select multiple scenes and/or scene collections based on their video needs. For example, multiple video sources can be accommodated

within a single scene. This can allow for preview of multiple video sources in tandem. Importantly, recordings in this configuration will include video from each source tiled within a single file **(Extended Figure 6-1A)**. Video from multiple sources can also be previewed across separate scenes within a single scene collection. However, OBS Studio is only able to record from one scene at any given time in a multiple scene configuration like this **(Extended Figure 6-1B)**. In instances when users want to record video from multiple sources in tandem into separate files, we recommend running multiple scene collections each with a single scene and video source in separate instances of OBS Studio **(Extended Figure 6-1C)**. To create one or more "scene collections" that can save any set of "scenes" and "video sources", go to the **Scene Collection** menu, and click **New (Figure 6A1)**. In the pop-up window, enter a name unique to your testing apparatus (e.g., "BOX1" for operant box 1) and click **OK** to finish creating the new scene collection **(Figure 6A2)**.

7. To add a video source to a scene collection, click on the **+** button on the **Sources** panel **(Figure 6A3)**, and select **Video Capture Device (Figure 6A4)**. In the pop-up window, enter a name (e.g., "BOX1 cam" for operant box 1 camera) for the new video source, and then click **OK (Figure 6A5)**. Once the "properties" window shows up, select the right camera source under **Device**, make additional changes to the device settings, and click **OK** to finish setting up the new video source **(Figure 6A6)**.
8. Click **Start Recording** on the **Controls** panel to start recording and **Stop Recording** to stop **(Figure 6A7)**.
9. For multi-camera recording in high throughput experiments (e.g., involving multiple subjects in separate testing sites), repeat **step 6-7** to create one "scene collection" for each camera. Then, launch one more instance of OBS Studio for each additional camera by double-clicking the desktop shortcut **(Figure 6B1)** followed by clicking **Launch Anyway** on the pop-up warning **(Figure 6B2)**. In each new instance, make sure

to select the “scene collection” that contains the correct camera source for the recording site of interest **(Figure 6B3)**. To confirm that the recording will not overload the computer CPU, monitor the CPU usage of each individual instance of OBS Studio provided in the lower right corner of the OBS interface **(Figure 6B4)** or in **Windows Task Manager**.

Frame rate analysis

Many neuroscience techniques, such as *in vivo* calcium imaging, combine video recordings with optical signal to correlate behavioral events with neural activity. The potential for dropped or duplicate frames, which we have experienced with commercially available USB webcams, poses non-trivial challenges for offline data analysis in these kinds of experiments. Recordings with dropped frames appear fast forward and have shorter duration during playback by third-party media players, which often assume a nominal frame rate that is higher than the actual frame rate achieved. Recordings with duplicate frames appear choppy, and thus have lower temporal resolution than desired, despite the high frame rate achieved. Both issues result in additional and unnecessary workload during post-processing in order to accurately align behavioral and neural data. To evaluate the frame rate performance of Pi USB Cam, videos of freely behaving adult male Long-Evans and Sprague Dawley rats were acquired from inside separate operant boxes (MED Associates, St Albans, VT, USA) using a Pi USB Cam and a generic commercial webcam (Logitech C930e webcam, Lausanne, Switzerland). Camera performance was assessed under both red and white house light illumination.

For dropped frame analysis, Synapse (Tucker-Davis Technologies TDT, Alachua, Fl, USA), an acquisition platform commonly used in neuroscience research, was used to acquire video recordings from both cameras simultaneously. Five videos of ~5 mins duration each were captured into AVI format in each lighting condition from each camera at the maximum resolution (640x480) and frame rate (20FPS) supported by Synapse. For each recording, the total number of frames and timestamps of each frame were read from Synapse data block using MATLAB

TDTbin2mat function (https://www.tdt.com/docs/sdk/offline-data-analysis/offline-data-matlab/overview/). For each video frame, Synapse stores two timestamps: one for the frame onset and one for offset. The onset timestamp of the very last frame was taken as the recording length, as the offset timestamp of the last frame was stored as *inf* instead of a real number. The true frame rate achieved was calculated using the following equation:

$$true\ FPS = \frac{total\ frames}{recording\ length\ (s)}$$

The total number of dropped frames was calculated as:

$$total\ dropped\ frames = target\ FPS \times recording\ length\ (s) - 1 - total\ frames$$

The number of dropped frames per minute was calculated as:

$$dropped\ frames\ per\ min = \frac{total\ dropped\ frames}{recording\ length\ (s)} \times 60$$

The video file length when played in third-party media players was calculated as:

$$video\ file\ length\ (s) = \frac{total\ frames}{target\ FPS}$$

For duplicate frame analysis, OBS Studio was used to acquire five videos of ~5 mins duration each into mp4 format in each lighting condition from each camera at 640x480 resolution and 30 FPS. Visual inspection of videos from each camera made clear that those acquired with the commercial webcam contained abundant duplicate frames, whereas those acquired with Pi USB Cam were essentially devoid of duplicate frames. To quantify this, a custom MATLAB script was used to extract relevant information from each recording, including the recording length and total number of frames, and to identify duplicate frames. Color video files were first converted to grayscale and the frame-by-frame difference in grayscale intensity was calculated for each pixel. Plotting the frequency distribution of maximum change in pixel intensity revealed a bimodal distribution apparent only in videos acquired with the commercial webcam. Using the local minimum as a guide, we identified a threshold for maximum pixel intensity change of 8 for videos acquired under red house light illumination and 10 for videos acquired under white house light illumination. Frames that contain a maximum pixel intensity change below or equal to these thresholds were algorithmically identified as duplicates, which matched the duplicates identified manually in videos acquired with the commercial webcam. The true frame rate of each video, which discounts the duplicate frames and more accurately reflects the temporal resolution, was calculated using the equation: $true\ FPS = \frac{total\ frames - total\ duplicate\ frames}{recording\ length\ (s)}$.

The number of duplicate frames per second was calculated as:

$$duplicate\ frames\ per\ min = \frac{total\ duplicate\ frames}{recording\ length\ (s)} \times 60.$$

Results were expressed as mean ± SEM.

Fisheye distortion correction

Fisheye camera lenses, such as the one used in the recommended Arducam day-night vision camera, allow for capture of a large field of view given a limited object distance. However, they often produce images that are radially distorted, which may pose challenges to accurate position tracking. Here, we describe a simple method to digitally correct fisheye image distortion using a free, open-source OBS Studio plugin, the OBS ShaderFilter.

Step-by-step instruction:

1. On a Windows PC installed with OBS Studio, download the latest release of OBS ShaderFilter plugin from its GitHub page: https://github.com/Oncorporation/obs-shaderfilter **(Extended Figures 8-1 A1-2)**, unzip the package file, and drag and drop its contents to the OBS program file directory **(Extended Figure 8-1 A3)**. The default file location is `C:\Program Files\obs-studio`. Replace duplicate files if necessary **(Extended Figure 8-1 A4)**.
2. Next, download the entire repository from its GitHub page and unzip **(Extended Figure 8-1 B1)**. Locate the "fisheye.shader" text file under the `obs-shaderfilter-master\data\examples` directory, drag and drop to the OBS program file directory at

`C:\Program Files\obs-studio\data\obs-plugins\obs-shaderfilter\examples` to store alongside other shader filters **(Extended Figure 8-1 B2)**.

3. Launch OBS Studio and add a video source if you have not **(Extended Figure 8-1 C1; Figure 6A)**. To import pre-recorded videos for offline fisheye correction, add a "Media Source" as the video source **(Extended Figure 8-1 C2)**. To configure a fisheye camera for real-time fisheye correction during recording, add a "Video Capture Device" as the video source **(Extended Figure 8-1 C2)**.
4. Right click the video source and select the "Filters" option **(Extended Figure 8-1 C3)**. In the pop-up window, under the "Effect Filters" header, click the "+" button to add a "User-defined shader" **(Extended Figure 8-1 C4)**, which can then be renamed to "Fisheye correction" for clarification **(Extended Figures 8-1 C5)**.

   NOTE: If the "User-defined shader" is missing, repeat **Steps 1 & 2** to make sure the plugin is correctly installed.
5. Select the option to "Load shader test from file" and click "Browse" to locate and load the "fisheye.shader" text file. Adjust the "power" parameter to enhance or reduce fisheye effect **(Extended Figure 8-1 C6)**.

   NOTE: A positive power adds more fisheye radial distortion while a negative power corrects distortion. A power of zero means no change to the original image. See **Extended Figure 8-2** for example power settings.
6. In the main OBS Interface, press "Start Recording" to record a new video if a camera has been selected as the source or re-record a video with fisheye distortion corrected if a pre-recorded video file has been selected as the source **(Extended Figure 8-1 C7)**.

Position tracking and locomotor activity measurement

To assess the effects of fisheye distortion and digital correction on locomotor activity measurement, position tracking was performed on videos with low distortion, fisheye distortion, and distortion digitally corrected. Five videos of ~5 mins duration of adult female Long-Evans rats exploring two contextually distinct compartments in a standard conditioned place preference apparatus (MED Associates, St Albans, VT, USA) were acquired in OBS Studio from two Pi USB Cams in tandem. One camera was equipped with a 70° HFOV low-distortion lens and the other with a 100° HFOV fisheye lens (Arducam Technology Co., Limited, Hong Kong, China; **Extended Table 1-1**). Videos with fisheye distortion were subsequently corrected using the method described above. Position and locomotor measures were tracked offline using ANY-maze software (Stoelting Co., Wood Dale, IL, USA). Each measure obtained was averaged across all five videos and compared using a one-way ANOVA.

For real-time position tracking, a Pi USB Cam was directly interfaced with ANY-maze as a USB camera to provide live video feed.

RESULTS

Pi USB Cam offers superior video quality & performance

Of significant importance to our research needs was an affordable solution that performed better under low-/no-light conditions within limited object distance compared to commercially available webcams. Pi USB Cam outperformed the Logitech C930e webcam on several measures of significance to behavioral neuroscience research. Monitoring of a standard rat operant testing apparatus with a working area of 11.625" L x 9.78" W x 7.35" H was successfully accomplished with the Pi USB Cam mounted overhead using the out-of-the-box 170° (DFOV) × 140° (HFOV) fisheye lens. Notably, in this orientation, the camera was placed less than 1 cm above the testing apparatus therefore requiring essentially no additional vertical space for video acquisition **(Figure 7A)**. In contrast, the commercial counterpart, equipped with a 90° DFOV lens, necessitated ~20 cm of vertical space to capture a similar field of view. Moreover, positioning of the Pi USB Cam allowed for unobstructed access to the roof opening enabling unimpeded movement of tethered animals, whereas angled placement of the Logitech webcam was required to avoid collision with the commutator and tether.

Camera performance was qualitatively accessed under no-visible light condition **(Figure 7B)**, and several standard low-visible light conditions inside an operant box including cue light illumination **(Figure 7C)**, red house light illumination **(Figure 7D)**, white house light illumination **(Figure 7E)**, and white house light + cue light illumination **(Figure 7F)**. With adequate IR illumination, Pi USB Cam provided high image quality regardless of the level of the ambient visible light. In contrast, the Logitech webcam was unable to perform in the no-light condition and image quality was compromised under red house light illumination.

Pi USB Cam also outperformed the commercial webcam in terms of frame rate maintenance. Unlike the Pi USB Cam, the commercial webcam was unable to maintain the set frame rate of 20 FPS when interfacing with Synapse, losing on average 505.60 ± 69.40 frames per min under white house light illumination and 702 ± 0.00 frames per min under red house light illumination **(Table 3)**. This resulted in video files that appear fast forward and shortened when played offline by third-party media players that assume a constant, nominal frame rate of 20 FPS **(Movie 1)**. Pi USB Cam, however, maintained a constant 20 FPS across all trials under both lighting conditions. In contrast to Synapse, OBS Studio was able to achieve the maximum frame rate of 30 FPS with either camera without dropping frames, as determined by the ratio of total number of captured frames to recording length (data not shown). However, videos recorded with the commercial webcam exhibited a high degree of duplicate frames. On average, the webcam contained 1300.20 ± 1.20 duplicate frames per min under white house light illumination and 1303.40 ± 0.24 duplicate frames per min under red house light illumination **(Table 4).** As a result, these videos appear choppy during playback reflecting reduced temporal resolution despite the high frame rate achieved **(Movie 2)**. Importantly, this analysis cannot distinguish between duplicate frames and instances when the animal does not move between two frames. However, it should be noted that videos acquired with Pi USB Cam were judged to be free of duplicate frames upon visual inspection. A similar algorithmic analysis of these videos failed to uncover a clear threshold suggestive of duplicates, as their frequency distributions of maximum change in grayscale pixel intensity appear unimodal as opposed to bimodal. Nevertheless, when the same thresholds used for the commercial webcam were applied to these videos, an average of 466.20 ± 43.24 frames per min were identified for videos under red house light illumination and 310.40 ± 100.74 frames per min for videos under white house light illumination. Not only was the number of identified frames much lower (64-76%) than that observed with the commercial webcam, but this value also varied greatly from video to video. Frames identified as duplicates were also unevenly distributed unlike those observed in videos acquired using the commercial webcam. Combined with visual inspection, these suggest that the algorithmically identified frames in Pi USB Cam videos reflect a lack of animal movement rather than duplicate frames.

Pi USB Cam is highly customizable for individual recording needs

The recommended Arducam day-night vision camera can produce high quality video images under both bright and low-/no-light conditions with relative ease **(Figures 7, 9-11, Movies 3-4)** and can also be permanently set to engage or disengage the IR filter **(Figure 3A**). Moreover, the ability to physically separate the accompanying IR LEDs from the camera body allows for flexibility in illumination options under low-/no-light conditions **(Figures 4, 10D-F)**.

We also benchmarked several M12 sized lenses compatible with the Arducam day-night vision camera **(**see list in **Extended Table 1-1)**, each of which is associated with a different field of view and accompanying degree of image distortion. Our research needs necessitate positioning of the Pi USB Cam off-center from the testing arena to accommodate for a tether accessed through the roof opening. However, this orientation has the potential to result in uneven image distortion across the field of view as distortion tends to be minimal at the center of the fisheye field of view and progressively enlarged toward the radial edge of the image (Clemmensson et al., 2020). Therefore, comparison of different lenses was performed with the camera placed directly above the center of the arena at the level of the plexiglass roof about 20 cm above the grid floor measuring 11.5" L x 10.25" D x 1.75" H. As shown in **Figure 8**, we found that the default lens (140° HFOV) struck the best balance between an adequate field of view to capture the majority of the area of interest and an acceptable amount of image distortion to enable accurate behavioral tracking. Comparison of the object distance required to obtain a similar field of view across different M12 fisheye lenses is provided in **Extended Figure 8-2**.

Using the default lens, Pi USB Cam was also able to capture a generous field of view when positioned for side viewing (at a distance of about 5 cm away from the plexiglass wall) **(Figure 9, Movie 3)**. However, in environments where object distance is not a limiting factor or position accuracy is a primary concern, users can opt for lenses with a narrower field of view and lower image distortion, or lenses designed specifically for minimum distortion **(**see **Extended Table 1-1)**. For example, overhead monitoring of a standard conditioned place preference testing apparatus with a working area of 32.70" L x 8.25" W x 8" H, we found that a 100° (HFOV) fisheye lens was ideally suited to capture the entire testing arena when the camera was positioned ~30 cm overhead **(Figure 11, Extended Figure 8-3, Movie 5-6)**.

Fisheye distortion correction allows for accurate position tracking and locomotor activity measurement

As an alternative to using low-distortion lenses, which often require larger object distance that is incompatible with many experimental set ups, fisheye distortion can be digitally corrected using the OBS ShaderFilter plugin **(Extended Figures 8-1, 8-2, and 8-3, Movie 5)** as well as other widely available image processing algorithms. Benchmarked against a low-distortion M12 lens, the fisheye image distortion from a 100 °HFOV lens and digital fisheye correction imparted minimal effects on position tracking efficiency **(Extended Figures 8-3 A-C, Movie 5)**. One-way ANOVA of various locomotor measures revealed a significant between group difference for distance travelled in the white side of the testing apparatus [$F(2,4) = 6.538$, $p = 0.0462$] **(Extended Figure 8-3 D)**. However, post-hoc comparisons failed to identify a significant difference between pre-correction and low-distortion (Dunnett's test, $p = 0.0918$) or post-correction and low-distortion (Dunnett's test, $p = 0.0783$). While no other statistically significant differences were observed, visual inspection of the data makes clear that the difference between pre-correction and low-distortion is lessened by digital correction. Therefore, digital fisheye correction is likely to improve tracking accuracy in experiments where absolute, rather than relative, measures are a high priority.

Custom 3D-printed components afford flexible installation options tailored to individual needs

Our custom 3D-printed components **(Table 2)** provide added protection and allow Pi USB Cam to be securely mounted on a variety of surfaces or structures. Using these components, Pi USB Cam can be installed on a commutator balance arm post **(Figures 9A, C)**, a ring stand **(Figure 10A)**, a wire shelf **(Figures 10D, 11A)**, or any flat surface **(Figures 9B, D)**. Moreover, the hinged camera mount components provide ample degrees of freedom to finely adjust camera positioning for optimal image acquisition. Thus, use of our custom 3D-printed components ensures Pi USB Cam is readily adaptable to virtually any recording condition. This includes recording within the limited space around a standard operant box inside a sound attenuating cabinet **(Figure 9, Movie 3)**, and many other common behavioral testing sites such as a home cage environment **(Figure 10, Movie 4)** or larger scale testing arenas like a conditioned place preference (CPP) apparatus **(Figure 11)**.

Pi USB Cam is highly scalable for multi-subject multi-site recording needs

Using the free, open-source software, OBS Studio, we were able to successfully video-monitor in real-time eight freely behaving rats in separate operant boxes with eight Pi USB Cams operating simultaneously from a single host computer **(Figure 12A)**. Acquired recordings were subsequently saved into individual video files for further offline analysis. Using our settings **(Figure 5D)**, a 29 min recording produced on average 738.5 MB worth of video file in mkv format (data not shown). At any given moment, each instance of OBS Studio used between 3-6% of the six-core 3.20GHz CPU on our host computer and the overall CPU usage never exceeded 50% when no other major application was running at the same time **(Figures 12B-C)**. Thus, the only major limiting factors to scaling Pi USB Cam are the USB bandwidth and CPU of the host computer.

DISCUSSION

Pi USB Cam is an affordable, DIY video recording solution that combines simple electronics and 3D printing to enable video monitoring of behavior in diverse recording environments under any lighting condition. Our detailed build instructions are easy to follow and require no specialized expertise. Camera components and accessories are easily sourced from globally accessible vendors. In addition, because DIY components are often discontinued, we provide an extensive list of alternatives that can serve as substitutes if circumstances require. Using this design, we demonstrate its ease of use as a plug-and-play USB camera, with notable superiority over a generic commercial webcam in terms of field of view, IR sensitivity, frame rate, and overall flexibility to meet individual research needs. Lastly, we show that Pi USB Cam, in combination with free, open-source video acquisition software, is easily scalable for multi-subject and multi-site recordings.

Hardware flexibility is the main advantage of Pi USB Cam over commercially available webcams. Indeed, despite their out-of-the-box user friendliness, the hardware of most commercial webcams is simply not designed to acquire high quality video recordings in confined spaces and/or poor lighting conditions. In contrast, Pi USB Cam can be used with a wide range of camera modules that offer IR sensitivity for low-light recording, IR correction for bright-light recording, fisheye lenses for wide-angle recording, low-distortion lenses for accurate position tracking, etc. Due to its DIY nature, the cost of building Pi USB Cam is comparable to, if not more affordable than, repurposed commercial webcams and a fraction of the cost of video capture systems specialized for neuroscience research **(Extended Table 1-2)**. This is particularly important given that camera costs can present significant challenges to scalability and throughput. Augmented by the burgeoning technology of 3D printing, Pi USB Cam can be installed in various commonly used behavioral testing and housing settings without the need for additional modifications to the testing apparatus or additional costly parts.

At the heart of the software design, the *Show-me webcam* firmware enables Raspberry Pi cameras to become an out-of-box, plug-and-play USB camera. In contrast, most, if not all, other DIY solutions require some level of programming proficiency to unlock even the most basic functionalities of the microcomputer and related hardware (Saxena et al., 2018; Singh et al., 2019; Weber and Fisher, 2019; Clemensson et al., 2020; Centanni and Smith, 2021). While DIY solutions tend to turn researchers away due to the steep learning curve associated with such requirements, Pi USB Cam ensures novices need not reinvent the wheel. As a USB camera, Pi USB Cam can be readily integrated into pre-existing video recording systems, ranging from the popular open-source recording/streaming app OBS Studio to the professional neural/behavioral recording software Synapse. As such, limited only by the specifications of the host recording computer, Pi USB Cam can be readily scaled to facilitate multi-subject, multi-site, high-throughput experimental designs. Depending on the recording software of choice, users of Pi USB Cam have the option to watch live video feeds for real-time behavioral monitoring on screen or via network streaming. Users also have the freedom to save video recordings in their preferred file format and location where they can be accessed for offline analysis using sophisticated behavioral tracking algorithms such as ezTrack (Pennington et al., 2019) and DeepLabCut (Mathis et al., 2018). For real-time applications that rely on live video feeds, Pi USB Cam is again able to integrate as a plug-and-play USB camera with powerful, yet widely available neuroscience research applications like ANY-maze, which combine real-time video tracking with various control devices (e.g., optogenetic laser) to achieve closed-loop neural/behavioral manipulation (see **Movie 6** for example). Although not tested here, Pi USB Cam is also compatible with recently developed open-source video tracking and control systems, which readily support USB cameras including DeepLabCut-Live (Kane et al., 2020) and DeepLabStream (Schweihoff et al., 2021).

While the Pi USB Cam offers an easy-to-use, flexible, and affordable option for behavioral neuroscientists to acquire video recordings, several limitations of our system should be noted. Previous approaches using Raspberry Pi cameras are designed such that the microcomputer performs the heavy lifting of video encoding (Saxena et al., 2018; Singh et al., 2019; Weber and Fisher, 2019; Clemensson et al., 2020; Centanni and Smith, 2021). The advantage of such a design is that it has no inherent limits on scalability other than the number of cameras an individual can purchase. In contrast, Pi USB Cam requires a standalone host computer to perform recordings. In addition to this expense and the footprint associated with a host computer in close proximity to behavioral testing apparatuses, the number of video streams that can be encoded and stored in tandem is limited by the host computer's processing power and bandwidth. While such a configuration could be problematic for some research needs, we felt that function similar to a commercial webcam makes Pi USB Cam more user friendly and approachable than standalone alternatives. Moreover, connection with a host computer provides significantly greater ease of real-time video monitoring than standalone Raspberry Pi cameras. While *Show-me webcam* is more than adequate for video recording, it does not currently support audio capture. Although this could change in a later release of the firmware, currently video recordings requiring audio measures (e.g., ultrasonic vocalizations, audio cues) necessitate use of a standalone microphone to capture audio independent of video feed, which can then be synchronized online or offline depending on the recording software used. As with any open-source DIY solution, Pi USB Cam software will require regular and timely updates in tandem with upgrades to Raspberry Pi hardware and operating system. However, the well-established open-source community of *Show-me webcam* developers has a strong history of providing regular support and upgrades for the firmware. This is unlike previous niche DIY video solutions, which have often relied on a single individual to perform upgrades and consequently have had difficulty surviving beyond initial hardware and operating system versions (Singh et al., 2019). Finally, our custom 3D-printed case and mount tools require access to a quality 3D printer. However, as the technology of 3D printing matures, small-scale consumer printers are becoming increasingly more affordable, and larger-scale industrial fee-for-service printers are often offered at universities/institutions.

In summary, Pi USB Cam is a highly versatile and affordable DIY video recording solution for real-time behavioral monitoring and offline analysis. Requiring minimum time, expertise, and financial commitment to implement, Pi USB Cam offers behavioral neuroscientists a powerful, yet simple, solution for high quality and high-throughput behavioral data collection. We encourage users to reference the current manuscript when implementing Pi USB Cam in their own experiments.

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FIGURE LEGENDS

Movie 1. Comparison of Pi USB Cam and commercial webcam performance using specialized neural recording software for video acquisition.

Movie 2. Comparison of Pi USB Cam and commercial webcam performance using OBS Studio

Movie 3. Example video showing versatile implementation of Pi USB Cam for behavioral monitoring.

Movie 4. Example video showing versatile implementation of Pi USB Cam for rodent home cage monitoring.

Movie 5. Example video showing position tracking performed on a video acquired with a fisheye lens before and after distortion correction, and a video obtained with a low-distortion lens.

Movie 6. Example video showing real-time closed-loop behavioral control by a commonly used video-tracking system combined with Pi USB Cam.

Figure 1. Camera software setup. (A) Insert a clean micro-SD card into a computer. **(B)** Download the latest release of the *Show-me webcam* image file from its official GitHub page. **(C)** Install the *Show-me webcam* image file on the micro-SD card using the official Raspberry Pi Imager. Image source: https://github.com/raspberrypilearning/components

Figure 2. Camera hardware setup. (A) Essential components required to build a Pi USB Cam including the main Raspberry Pi board, a USB to USB-C cable, a micro-SD card, the camera module, and a camera ribbon flex cable (listed from left to right). **(B)** Remove the attached IR LEDs from the main camera board by unscrewing the four screws outlined in red. **(C)** Connect the main camera board to the Raspberry Pi 4B board via a camera ribbon flex cable. See Extended Figure 2-1 for a labeled diagram of the Pi motherboard. **(D)** Insert the prepared micro-SD card (see Figure 1 for software setup) into the Raspberry Pi board. **(E)** Power up the Pi USB Cam by connecting it to a computer using the USB cable. **(F)** House the camera in custom 3D-printed case and mount tools for protection and installation in behavioral testing environment.

Figure 3. Adjusting camera settings. (A) Manually enable IR sensitivity by covering the photoresistor with non-translucent tape **(1)** or enable IR correction by unplugging the connector on the back of the camera **(2)**. **(B)** Use of our recommended low-light custom settings (right) eliminates slightly purple hue obtained using the camera's default settings (left) when recording in total darkness. **(C)** In contrast, the same low-light settings produce an unwanted yellow hue (right) under bright light conditions when default settings (left) produce a more appropriate image. **(D)** To adjust camera settings, connect the Pi USB Cam containing the micro-SD card into a host computer. **(E)** Identify the COM port for the Pi USB Cam in Windows Device Manager. **(F)** Connect to the Pi USB Cam using the open-source software PuTTY. **(G)** Enter the command shown in **(1)** to access all adjustable camera setting **(2)**. Settings we recommend adjusting for low-light recordings are highlighted in red. **(H)** Adjust the focus by unscrewing the small screw that secures the lens in place (arrow) and twisting the lens in either direction until your desired image quality is achieved. *Recommended settings for each lighting condition.

Figure 4. Wiring IR LEDs independent from Pi USB Cam. (A) Unwanted flare artifacts are apparent when IR LED boards remain attached to and powered directly by the camera board

and the camera is mounted over a reflective surface. Detaching the IR LEDs from the camera board allows for flexible placement in a configuration that avoids these flares as well as shadows cast by other equipment such as what is observed when the LEDs are mounted overhead **(B)** versus away from the reflective surface **(C)**. **(D)** Custom-made jumper wires **(1-3)** necessary to wire the IR LED boards in parallel and power them via a pair of 3v3 power and ground pins on the Pi board. See Extended Figure 4-1 and 4-2 for additional wiring instructions. **(E)** Fully connected IR LED unit and Pi board. The locations of 3v3 power pins (highlighted in pink) and ground pins (highlighted in in black) on the 40-pin header are illustrated in the inset. **(F)** Heat sinks should be installed on the back of the LED boards **(1)** to prevent overheating after housing them in the custom 3D printed case **(2)**. **(G)** CAD rendering of the IR LED housing and mount parts **(1)** and a close-up photo of a fully assembled and mounted IR LED unit **(2)**.

Figure 5. Set up for multi-camera recordings. (A) The following steps should be done in OBS Studio from a host computer connected to one or more Pi USB Cams. **(B)** Verify in the Windows Device Manager that no more than three cameras are connected to each USB controller. **(C)** Main OBS Studio interface. Menu, Sources, and Controls panels are highlighted. Users can generate a "Profile" from the drop-down menu **(D1-2)** and use the "Controls" panel to access various recording settings **(D3-7)**. Image source: https://github.com/obsproject/obs-studio/blob/master/UI/forms/images/obs_256x256.png

Figure 6. Recommended configuration for separate recordings from multiple video sources. For each Pi USB Cam, create a "Scene Collection" from the drop-down menu **(A1-2)** and add the camera as a video source using the "Sources" panel **(A3-7)**. **(B)** Launch additional instances of OBS Studio to record from multiple camera sources. See Extended Figure 6-1 for a detailed explanation of OBS Studio configurations.

Figure 7. Video quality comparison between Pi USB Cam and commercial webcam. Snapshots of videos acquired using Pi USB Cam (left column) and Logitech C930e webcam (right column) mounted overhead at a distance that accommodated the entire field of view **(A)**. Recordings were made under **(B)** no light, **(C)** cue light illumination, **(D)** red light illumination, **(E)** white light illumination, and **(F)** white light illumination + cue lights.

Figure 8. Comparison of different Pi USB Cam-compatible fisheye lenses. Pictures of a Pi USB Cam equipped with various M12 fisheye lenses **(1)** including 180° **(A)**, 140° **(B)**, 118° **(C)**, 100° **(D)**, 73° **(E)**, 67° **(F)**, 33° **(G)**, and 26° **(H)**. Corresponding snapshots of videos acquired from a centered position overhead of the arena at a distance of ~20 cm from the rod floor are depicted for each lens **(2)**. *Indicates the default lens that comes with the Arducam day-night vision camera. See accompanying Extended Figures for digital fisheye distortion correction **(8-1)**, comparison of lenses at different object distances **(8-2)**, and position tracking comparisons under distorted and low-distortion video acquisition settings **(8-3)**.

Figure 9. Camera implementation for operant box monitoring. Use of custom 3D-printed components allows for versatile camera installation options including overhead **(A-B)** and side **(C-D)** mounted configurations. For each option, **(1)** depicts the CAD drawing of 3D printed components. Close-up photos of each configuration are provided in **(2)** with object distance indicated in **(3)**. For overhead viewing, Pi USB Cam can be mounted on a commutator balance arm post **(A)** or directly on the roof panel **(B)**. Similar post **(C)** and wall **(D)** mount options are available for side view configuration. Both overhead **(E)** and side view **(F)** configurations allow for full view of the operant arena. Video snapshots acquired under no-light conditions.

Figure 10. Camera implementation for home cage monitoring. Custom 3D-printed components can also be configured to accommodate home cage recordings in a variety of

settings including mount to a ring stand **(A-C)** for recordings performed offsite (e.g., testing room) and on wire shelving **(D-F)** for recordings inside the vivarium. For each option **(1)** depicts the CAD drawing of 3D printed components. Close up photos of each configuration are provided in **(2)** with object distance indicated in **(3)**. Pi USB Cam is easily mounted to a ring stand **(A)** to accommodate offsite recordings in the home cage shown here in both bright-light **(B)** and no-light **(C)** conditions. Note that in this configuration the IR LEDs are used in the default configuration such that they are attached to and powered directly by the main camera board. Using the custom 3D-printed G clamp, Pi USB Cam (and independent IR LEDs) can also be mounted directly to shelving **(D)** shown here in both bright-light **(E)** and no-light **(F)** conditions.

Figure 11. Camera configuration for large apparatus video recordings. Together with custom 3D-printed components, Pi USB Cam can be configured to record from large behavioral testing arenas like the conditioned place preference apparatus shown here. **(A1)** CAD drawing of the 3D printed components used in this configuration. **(A2)** Close-up photo of Pi USB Cam mounted overhead on a wire shelf. **(A3)** The entire testing arena is visualized overhead with the camera mounted at a distance of ~30 cm using a 100° HFOV fisheye lens. Video snapshots acquired with this configuration under bright-light **(B)** and no-light **(C)** conditions.

Figure 12. Pi USB Cam scalability is limited only by USB bandwidth and host computer specifications. (A) Multi-subject multi-site recordings are easily achieved using the free and open-source video capture software OBS Studio depicted here with eight independent Pi USB Cams installed in eight separate operant boxes. **(B)** Windows Task Manager shows the CPU usage of each instance of OBS Studio during multi-camera recordings (n=8) using the same video acquisition settings shown in Figure 5D and a host computer with the specifications depicted in **(C)** including CPU, memory **(1)**, and add-on USB controllers from a USB PCIe expansion card **(2)**.

Table 1. Store-bought components list. Vendor, catalog number and price for all store-bought components used in current build. See Extended Table 1-1 for a comprehensive list of parts plus alternative options and additional accessories. See Extended Table 1-2 for cost estimates for complete build.

Table 2. Custom 3D-printed components. Comprehensive list of all 3D-printed components used in the current build. STL files and print instruction are accessible at https://www.thingiverse.com/gloverlab/designs.

Table 3. Dropped frame comparison between Pi USB Cam and commercial webcam. 5-minute videos (640x480 resolution, 20 FPS, avi format) were recorded from each camera via Synapse software for five trials under red and white house light illumination. Results are expressed as mean ± SEM.

Table 4. Duplicate frame comparison between Pi USB Cam and commercial webcam. 5-minute videos (640x480 resolution, 30 FPS, mp4 format) were recorded from each camera via OBS Studio for five trials under red and white house light illumination. Results are expressed as mean ± SEM.

Extended Figure 2-1. Diagram of the Raspberry Pi 4B motherboard. Image source: https://github.com/raspberrypi/documentation/blob/develop/documentation/asciidoc/computers/os/using-gpio.adoc

Extended Figure 4-1. Step-by-step instructions for crimping ring terminal connectors. (A) Gather all essential parts: **(1)** electrical wire AWG22, non-insulated ring terminal connector

AWG22-16 #4 stud size, heat shrink tubing 1/8”, **(2)** Astro crimping tool with B-Jaw for non-insulated terminals, and wire stripper. **(B-D)** Strip the wire to expose ~4 mm of conducting wire, comparable to the length of the wire barrel of the connector. **(D)** Cut one short segment of 1/8” heat shrinking tubing that is long enough to cover the entire wire barrel (≥ 6 mm) and put it on the wire before crimping. **(E)** Crimp the connector to the bare wire using the first die (DIN 0.5-1.5mm^2) on the B-Jaw of the Astro crimping tool. **(F)** Make sure to visually inspect and perform a gentle pull test to confirm the crimp is successful. **(G)** Fully cover the entire wire barrel with heat shrink tubing and use a heat gun to shrink the tubing.

Extended Figure 4-2. Step-by-step instructions for crimp pin terminal connectors. (A) Gather all essential parts: **(1)** electrical wire AWG22, male or female pin terminal connector, plastic housing, **(2)** Astro crimping tool with H-Jaw for open barrel terminals, and wire stripper. **(B-C)** Strip the wire to expose ~2.5mm of conducting wire. **(D)** Insert the stripped wire into the pin terminal connector while making sure that the bare wire falls within the wire barrel of the connector and the wire insulation is inside the insulation barrel. Gently bend the insulation barrel around the wire insulation to prevent connector or wire from moving out of place during crimping. **(E)** Crimp the connector to the wire using the middle die (DIN 0.5mm2) on the H-Jaw of the Astro crimping tool. Make sure that the open barrel is facing towards the “nest” of the crimping die and both wire barrel and insulation barrel are positioned in the appropriate spots of the crimping die. **(F)** Visually inspect and perform a gentle pull test to confirm the crimp is successful. **(G)** Insert the crimped connector into a plastic housing. Visually inspection and gentle pull test should be performed to make sure that the connector is securely housed in order to avoid issues with mating pins.

Extended Figure 6-1. Schematics showing several options to preview and/or record from multiple video sources. (A) One scene with multiple video sources operating within one instance of OBS Studio recorded into a single video file. **(B)** Multiple scenes each with their own video source operating within one instance of OBS Studio. Recording produces video from a single scene / video source. **(C)** Multiple scene collections each with their own video source running in independent instances of OBS Studio produce independent video files. Image source: https://github.com/obsproject/obs-studio/blob/master/UI/forms/images/obs_256x256.png

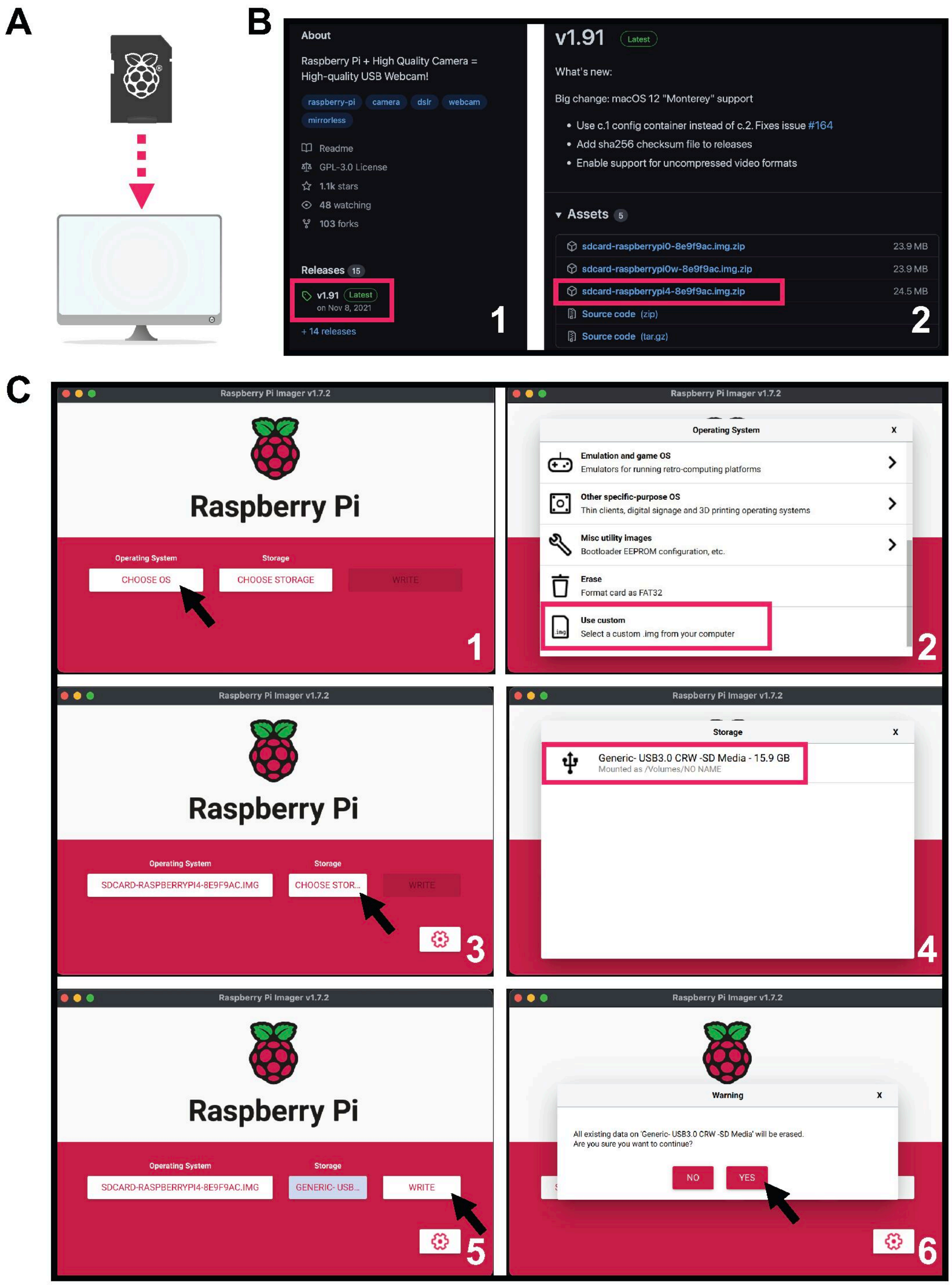
Extended Figure 8-1. Digital correction of fisheye distortion. (A) Download the OBS ShaderFilter plugin from its GitHub page and install on a Windows PC equipped with OBS Studio. **(B)** Download the "fisheye.shader" text file located in its GitHub repository. In the main OBS interface, add a pre-recorded video as a "Media source" for offline fisheye correction or a camera as a "Video Capture Device" for real-time fisheye correction **(C1-2)**. Enable the ShaderFilter plugin and fisheye correction for each video source **(C3-7)**.

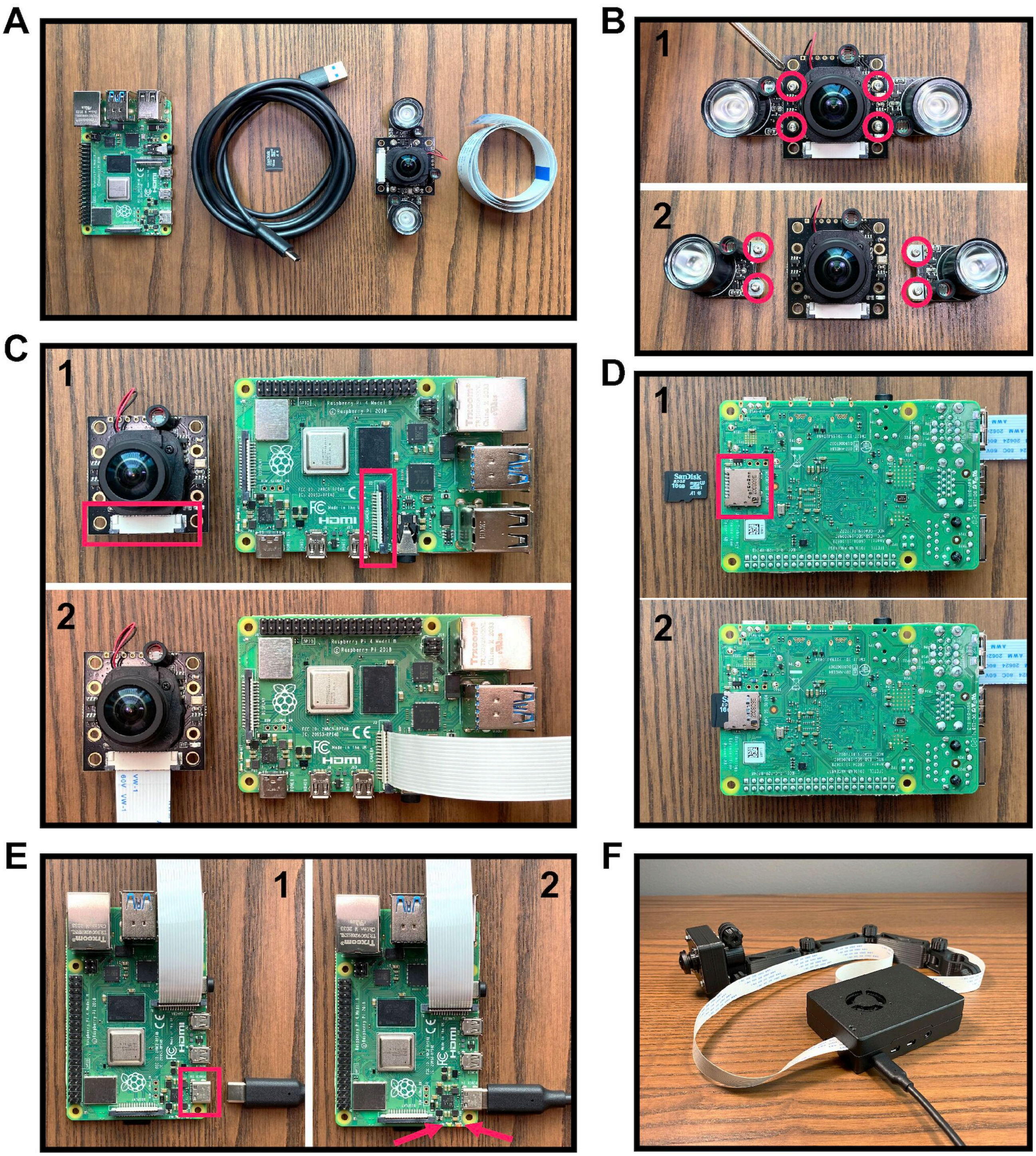
Extended Figure 8-2. Comparison of Pi USB Cam-compatible fisheye lenses in terms of object distance and fisheye correction. Pictures showing the object distance of a Pi USB Cam **(1)** equipped with various M12 fisheye lenses including 180° **(A)**, 140° **(B)**, 118° **(C)**, 100° **(D)**, and 73° **(E)** for overhead viewing in a standard operant box. Snapshots of raw videos acquired from a centered position at the corresponding object distances showing comparable captured fields **(2)**, and the same videos after the fisheye image distortion was digitally corrected using the OBS ShaderFilter plugin **(3)**. Power settings used are indicated on each image. *Indicates the default lens that comes with the Arducam day-night vision camera.

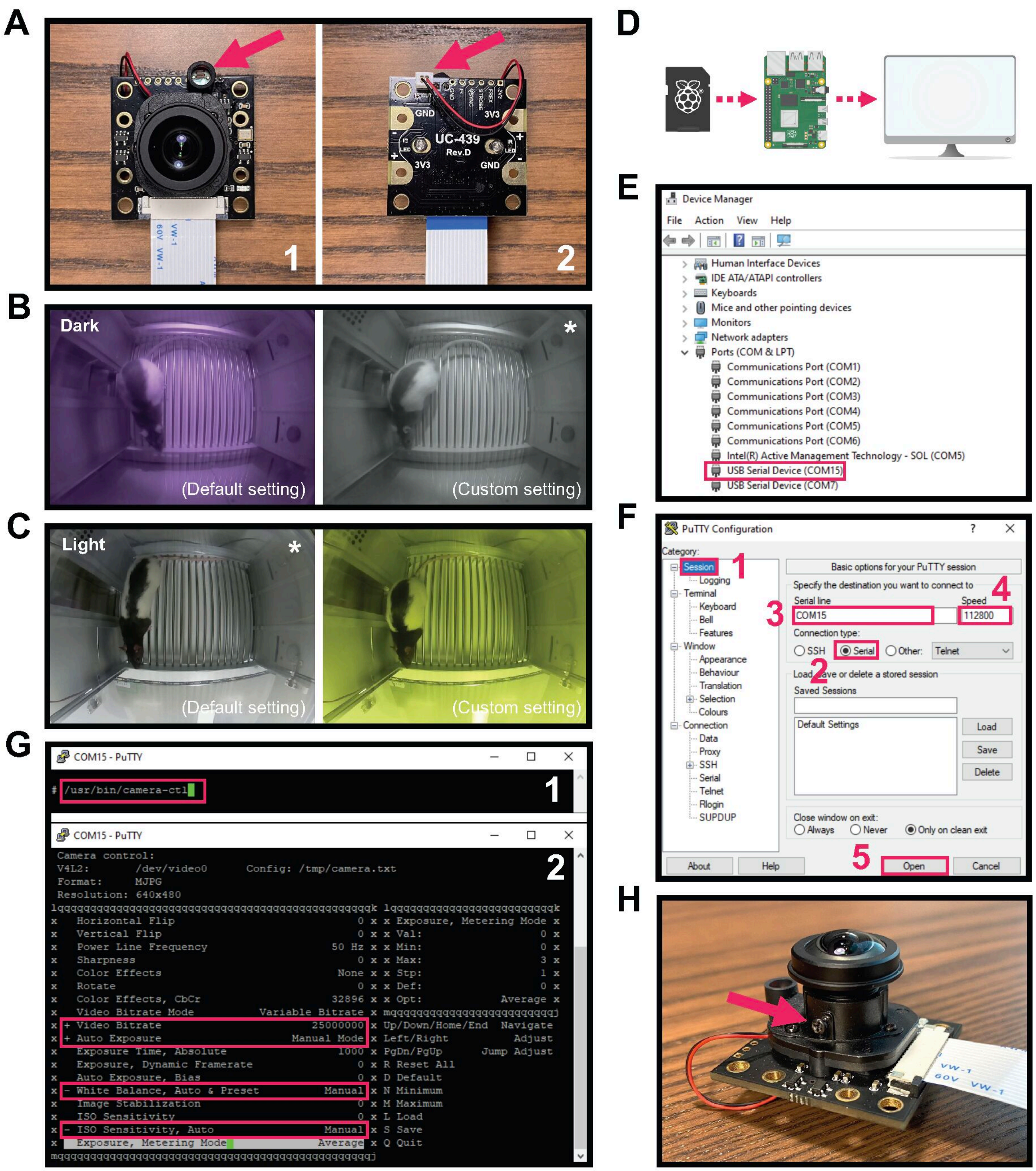
Extended Figure 8-3. Effects of fisheye distortion on position tracking and locomotor measures. Snapshots of ANY-maze position tracking **(1)** performed on a video acquired with a 100° HFOV fisheye lens without distortion correction **(A)**, after digital distortion correction **(B)**, and from a video acquired in tandem using a 70° HFOV low-distortion lens **(C)**. Representative center-point tracking plots **(2)** and heatmaps **(3)** from the same video show similar results. **(D)** Effects of fisheye distortion and correction on position tracking accuracy were demonstrated by comparing various locomotor activity measures, including the total distance travelled **(1)** and average speed **(2)** during the entire test duration, as well as the total distance travelled **(3-4)**, average speed **(5-6)**, and time spent **(7-8)** in either side of the testing apparatus.

Extended Table 1-1. List of store-bought components including alternative options and accessories.

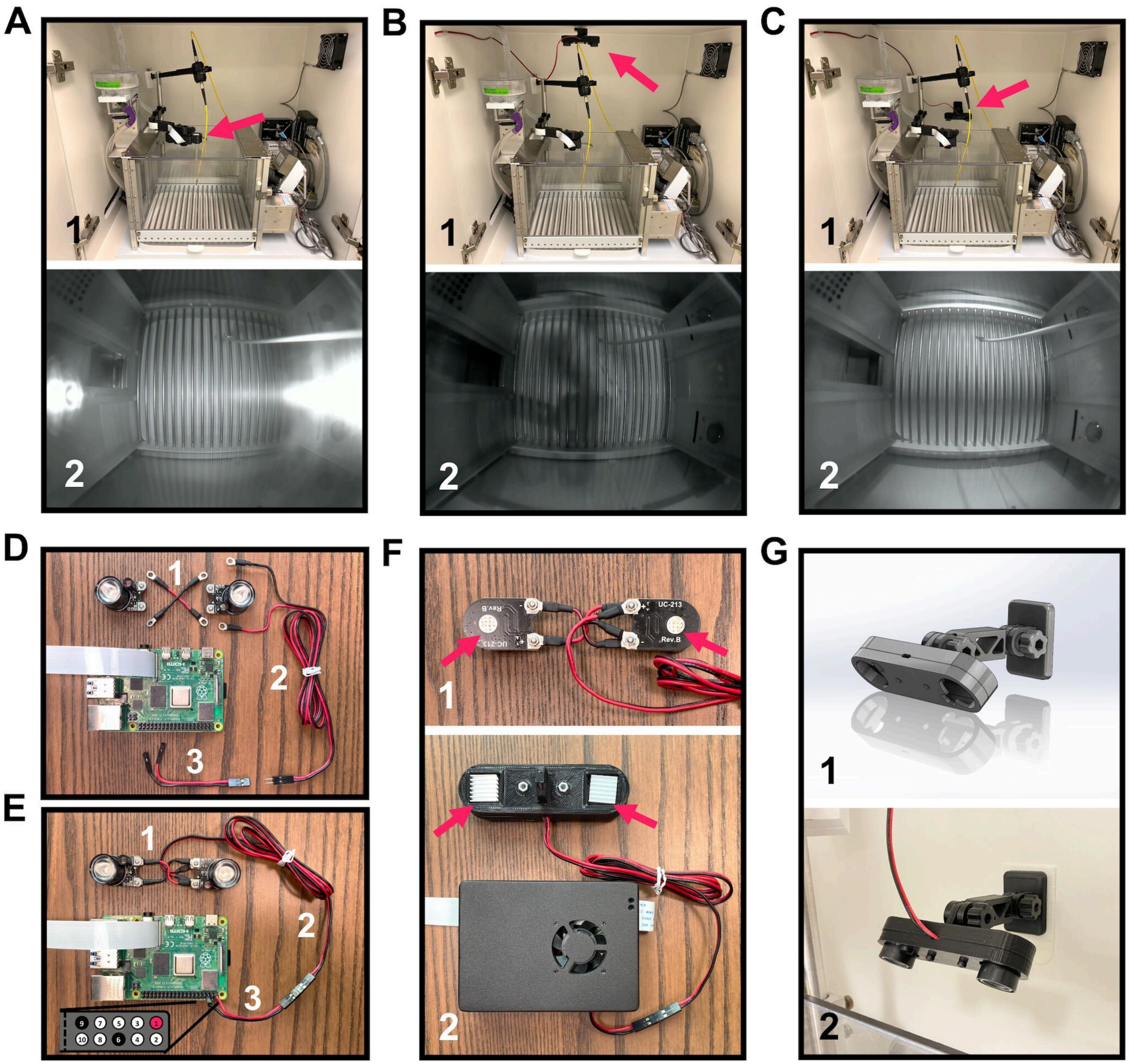
Extended Table 1-2. Pi USB Cam Expenses. Laboratories can choose equipment based on budget constraints. Tools and supplies are listed separately because they are only required if users intend to use the IR LEDs independently from the camera as shown in Figure 4 and/or if laboratories are not already stocked with these items. Note that the grand total (n=8) for the upper bound includes the cost of one set of tools and supplies as well as the cost for eight sets of essential equipment. Our university rate for 3D-printing services of $30/kg of PLA plastic was used to calculate the upper bound cost of 3D printed components, whereas the lower bound was calculated as the expense for only the purchase of plastic for those laboratories that already have a 3D printer. A complete camera (Figure 2F) and IR LED (Figure 4G) holder is estimated to require ~130 g of PLA plastic based on our recommended print settings. Commercial Webcam = Logitech C930e Webcam; Industrial camera estimates based on quotes from June 2022 from three widely used behavioral neuroscience suppliers.


A
B
About
Raspberry Pi + High Quality Camera = High-quality USB Webcam!
raspberry-pi
camera
dslr
webcam
mirrorless
Readme
GPL-3.0 License
1.1k stars
48 watching
103 forks
Releases 15
v1.91 Latest
on Nov 8, 2021
+ 14 releases
1
v1.91 Latest
What's new:
Big change: macOS 12 "Monterey" support
Use c.1 config container instead of c.2. Fixes issue #164
Add sha256 checksum file to releases
Enable support for uncompressed video formats
Assets 5
sdcard-raspberrypi0-8e9f9ac.img.zip 23.9 MB
sdcard-raspberrypi0w-8e9f9ac.img.zip 23.9 MB
sdcard-raspberrypi4-8e9f9ac.img.zip 24.5 MB
Source code (zip)
Source code (tar.gz)
2
C
Raspberry Pi Imager v1.7.2
Raspberry Pi
Operating System
Storage
CHOOSE OS
CHOOSE STORAGE
WRITE
1
Operating System
Emulation and game OS
Emulators for running retro-computing platforms
Other specific-purpose OS
Thin clients, digital signage and 3D printing operating systems
Misc utility images
Bootloader EEPROM configuration, etc.
Erase
Format card as FAT32
Use custom
Select a custom .img from your computer
2
SDCARD-RASPBERRYPI4-8E9F9AC.IMG
CHOOSE STOR...
3
Storage
Generic- USB3.0 CRW -SD Media - 15.9 GB
Mounted as /Volumes/NO NAME
4
GENERIC- USB...
5
Warning
All existing data on 'Generic- USB3.0 CRW -SD Media' will be erased.
Are you sure you want to continue?
NO
YES
6

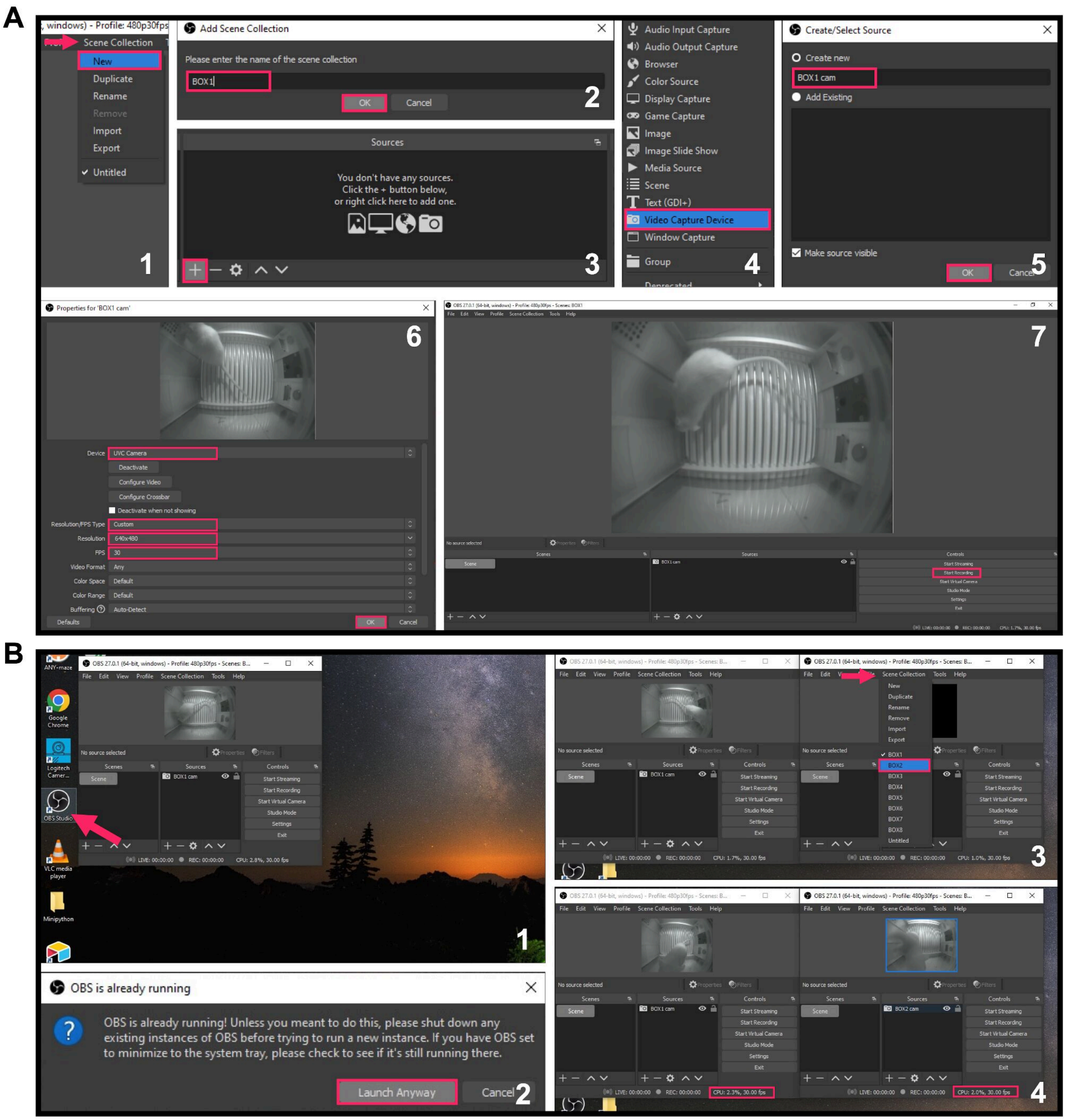

A
B
1
2
C
1
2
D
1
2
E
1
2
F



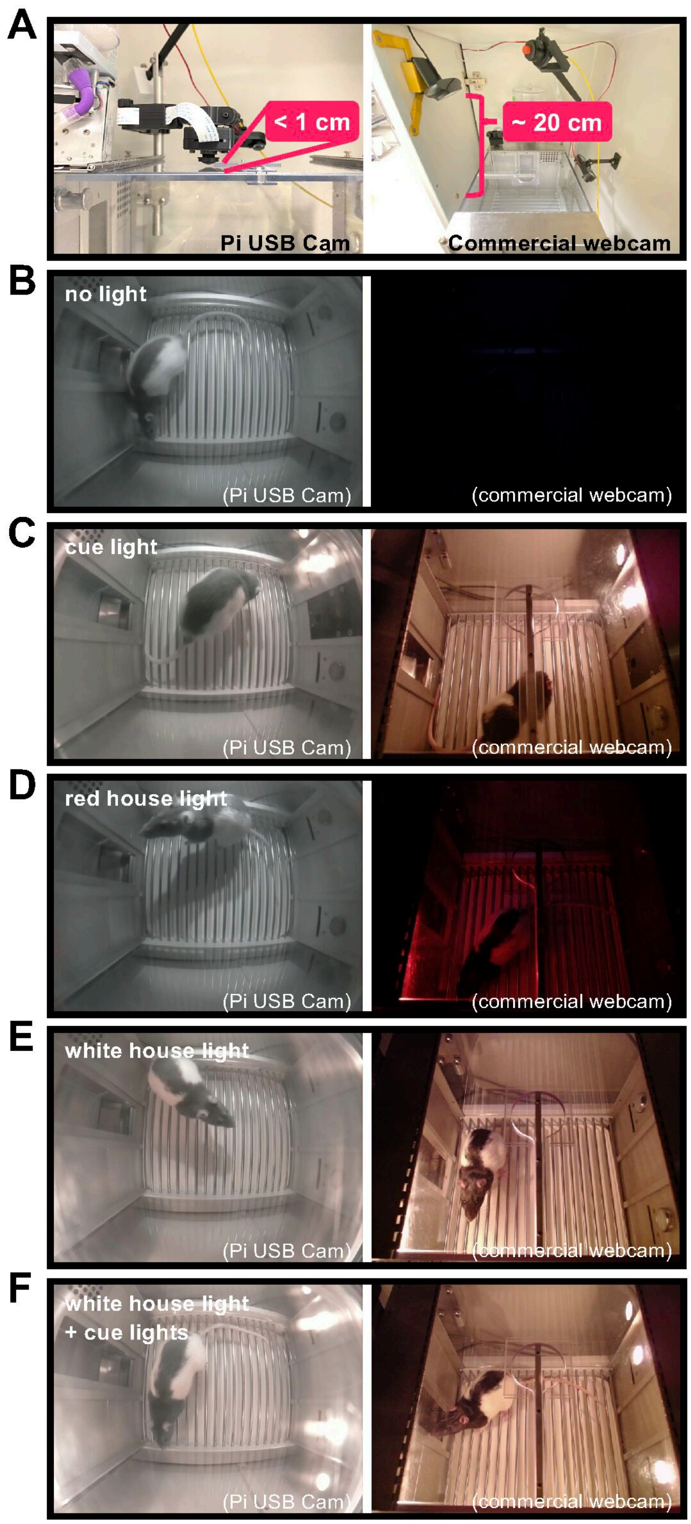
A
1
2
UC-439
Rev.D
GND
3V3
B
Dark
*
(Default setting)
(Custom setting)
C
Light
*
(Default setting)
(Custom setting)
D
E
Device Manager
File Action View Help
Human Interface Devices
IDE ATA/ATAPI controllers
Keyboards
Mice and other pointing devices
Monitors
Network adapters
Ports (COM & LPT)
Communications Port (COM1)
Communications Port (COM2)
Communications Port (COM3)
Communications Port (COM4)
Communications Port (COM5)
Communications Port (COM6)
Intel(R) Active Management Technology - SOL (COM5)
USB Serial Device (COM15)
USB Serial Device (COM7)
F
PuTTY Configuration
Category:
Session
Logging
Terminal
Keyboard
Bell
Features
Window
Appearance
Behaviour
Translation
Selection
Colours
Connection
Data
Proxy
SSH
Serial
Telnet
Rlogin
SUPDUP
Basic options for your PuTTY session
Specify the destination you want to connect to
Serial line
Speed
COM15
112800
Connection type:
SSH
Serial
Other:
Telnet
Load, save or delete a stored session
Saved Sessions
Default Settings
Load
Save
Delete
Close window on exit:
Always
Never
Only on clean exit
About
Help
Open
Cancel
1
2
3
4
5
G
COM15 - PuTTY
/usr/bin/camera-ctl
1
2
Camera control:
V4L2: /dev/video0 Config: /tmp/camera.txt
Format: MJPG
Resolution: 640x480
Horizontal Flip 0
Vertical Flip 0
Power Line Frequency 50 Hz
Sharpness 0
Color Effects None
Rotate 0
Color Effects, CbCr 32896
Video Bitrate Mode Variable Bitrate
+ Video Bitrate 25000000
+ Auto Exposure Manual Mode
Exposure Time, Absolute 1000
Exposure, Dynamic Framerate 0
Auto Exposure, Bias 0
- White Balance, Auto & Preset Manual
Image Stabilization 0
ISO Sensitivity 0
- ISO Sensitivity, Auto Manual
Exposure, Metering Mode Average
Exposure, Metering Mode
Val: 0
Min: 0
Max: 3
Stp: 1
Def: 0
Opt: Average
Up/Down/Home/End Navigate
Left/Right Adjust
PgDn/PgUp Jump Adjust
R Reset All
D Default
N Minimum
M Maximum
L Load
S Save
Q Quit
H


A
B
C
D
E
F
G
1
2
3
UC-213
Rev.B


A
B
Device Manager
File
View
Help
1
Devices by type
Devices by connection
Devices by container
Resources by type
Resources by connection
Show hidden devices
Customize...
Mice and other pointing devices
Device Manager
File Action View Help
2
DESKTOP-D142SNK
ACPI x64-based PC
Microsoft ACPI-Compliant System
ACPI Fixed Feature Button
ACPI Power Button
High precision event timer
Intel(R) Xeon(R) CPU W3670 @ 3.20GHz
Microsoft Windows Management Interface for ACPI
PCI Bus
High Definition Audio Controller
Intel Chipset SATA RAID Controller
Intel(R) ICH10 Family USB Enhanced Host Controller - 3A3A
USB Root Hub
USB Composite Device
USB Serial Device (COM8)
UVC Camera
USB Serial Device (COM11)
USB Serial Device (COM12)
Intel(R) ICH10 Family USB Enhanced Host Controller - 3A3C
USB Serial Device (COM10)
USB Serial Device (COM3)
PCI-to-PCI Bridge
3
ASMedia USB 3.1 eXtensible Host Controller - 1.10 (Microsoft)
USB Root Hub (USB 3.0)
USB Serial Device (COM6)
USB Serial Device (COM7)
USB Serial Device (COM9)
C
Menu
Sources
Controls
D
Profile
Scene Collection
New
Duplicate
Rename
Remove
Import
Export
Untitled
1
Add Profile
Please enter the name of the profile
480p30fps
Show auto-configuration wizard
OK
Cancel
2
Controls
Start Streaming
Start Recording
Start Virtual Camera
Studio Mode
Settings
Exit
3
Settings
General
Stream
Output
Audio
Video
Hotkeys
Advanced
Output Mode Simple
Streaming
Video Bitrate 2500 Kbps
Encoder Software (x264)
Audio Bitrate 160
Enable Advanced Encoder Settings
Recording
Recording Path
Browse
Generate File Name without Space
Recording Quality High Quality, Medium File Size
Recording Format mkv
Encoder Software (x264 low CPU usage preset, increases file size)
Custom Muxer Settings
Enable Replay Buffer
4
Base (Canvas) Resolution 640x480
Output (Scaled) Resolution 640x480
Aspect Ratio 4:3
Downscale Filter Bicubic (Sharpened scaling, 16 samples)
Common FPS Values 30
5
Filename Formatting %CCYY-%MM-%DD %hh-%mm-%ss
Overwrite if file exists
Automatically remux to mp4
Replay Buffer Filename Prefix Replay
Suffix
6
OK
Cancel
7



A
Scene Collection
New
Duplicate
Rename
Remove
Import
Export
Untitled
1
Add Scene Collection
Please enter the name of the scene collection
BOX1
OK
Cancel
2
Sources
You don't have any sources.
Click the + button below,
or right click here to add one.
3
Audio Input Capture
Audio Output Capture
Browser
Color Source
Display Capture
Game Capture
Image
Image Slide Show
Media Source
Scene
Text (GDI+)
Video Capture Device
Window Capture
Group
4
Create/Select Source
Create new
BOX1 cam
Add Existing
Make source visible
OK
5
6
7
B
1
OBS is already running
OBS is already running! Unless you meant to do this, please shut down any existing instances of OBS before trying to run a new instance. If you have OBS set to minimize to the system tray, please check to see if it's still running there.
Launch Anyway
Cancel
2
3
4


A
< 1 cm
~ 20 cm
Pi USB Cam
Commercial webcam
B
no light
(Pi USB Cam)
(commercial webcam)
C
cue light
(Pi USB Cam)
(commercial webcam)
D
red house light
(Pi USB Cam)
(commercial webcam)
E
white house light
(Pi USB Cam)
(commercial webcam)
F
white house light
+ cue lights
(Pi USB Cam)
(commercial webcam)

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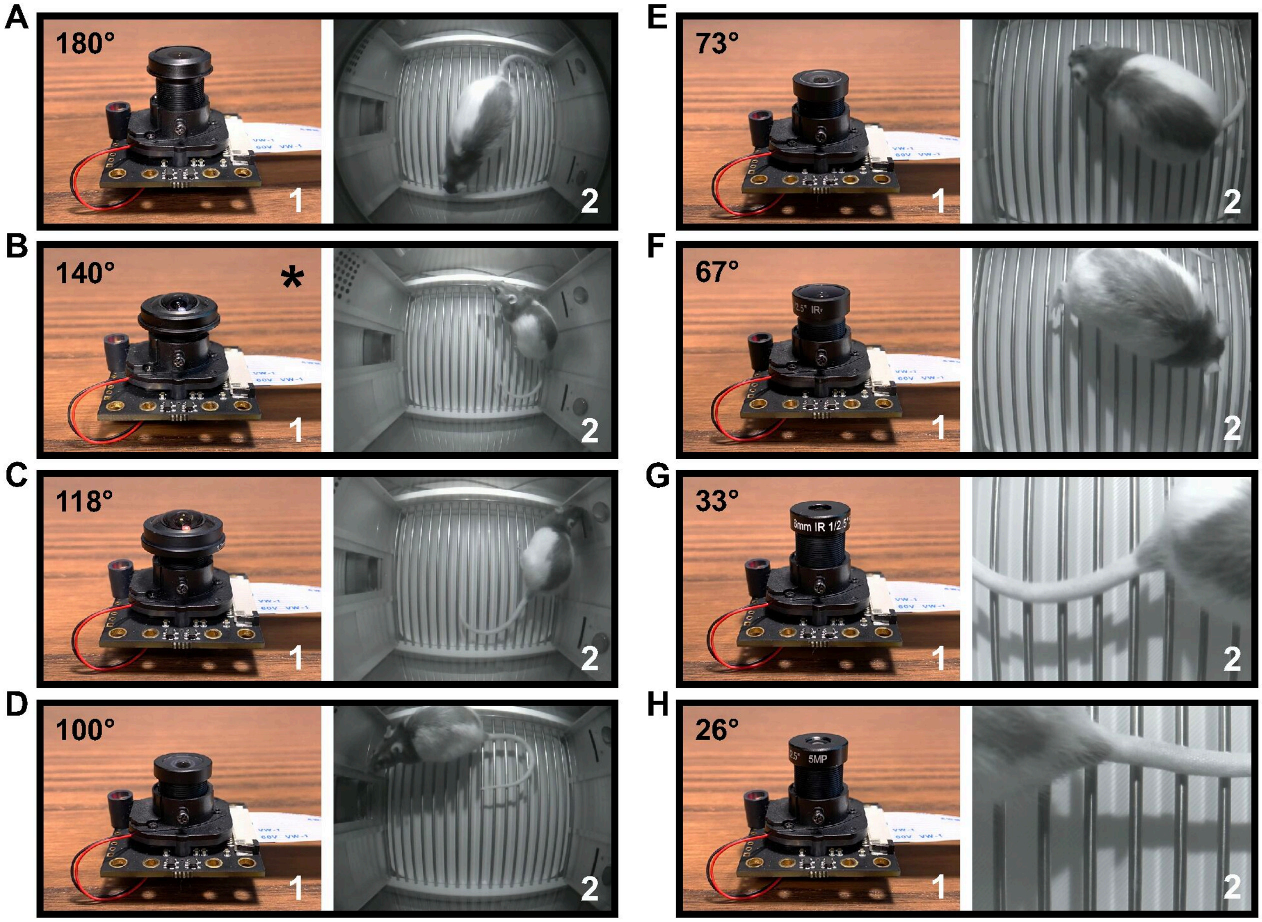
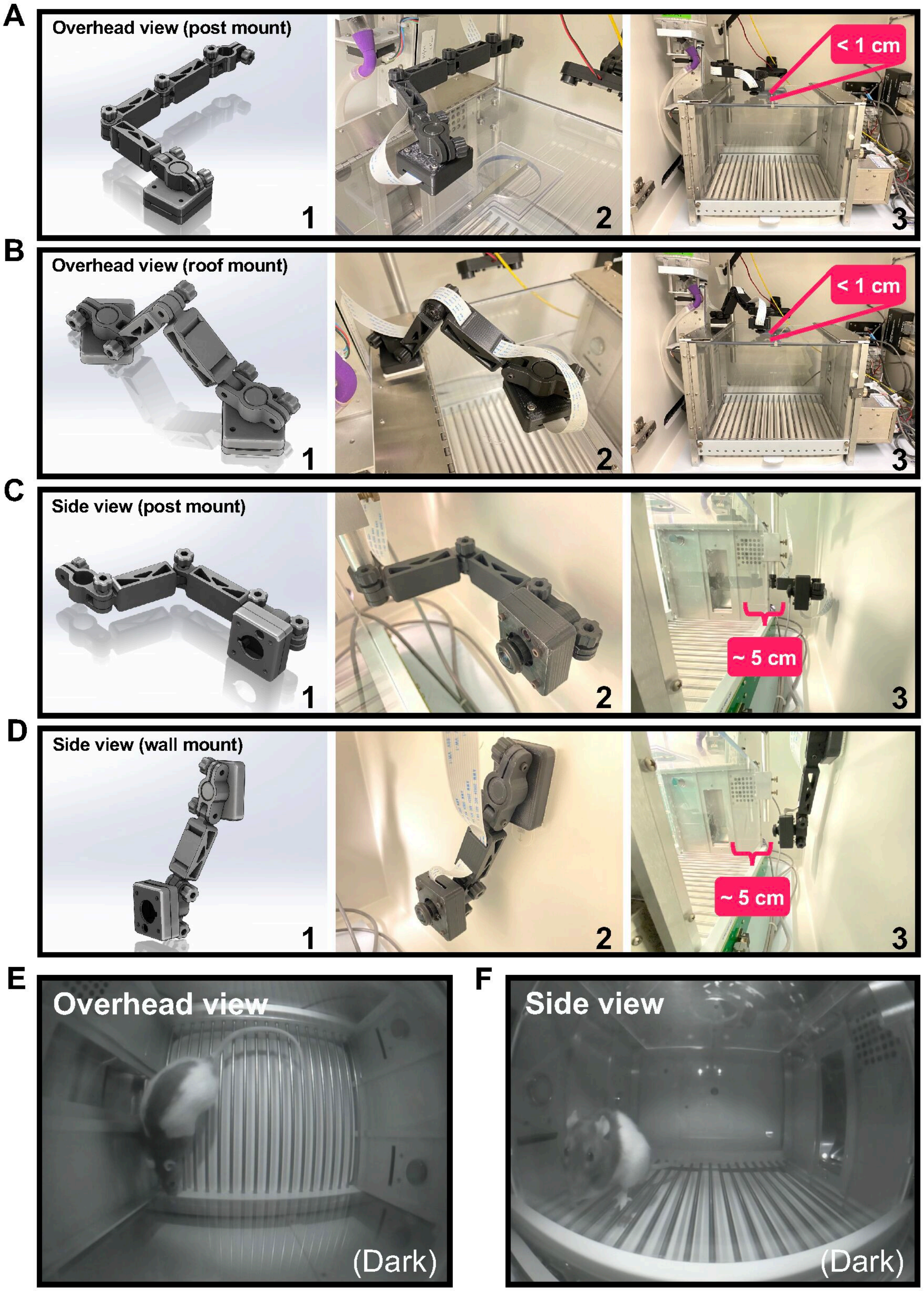
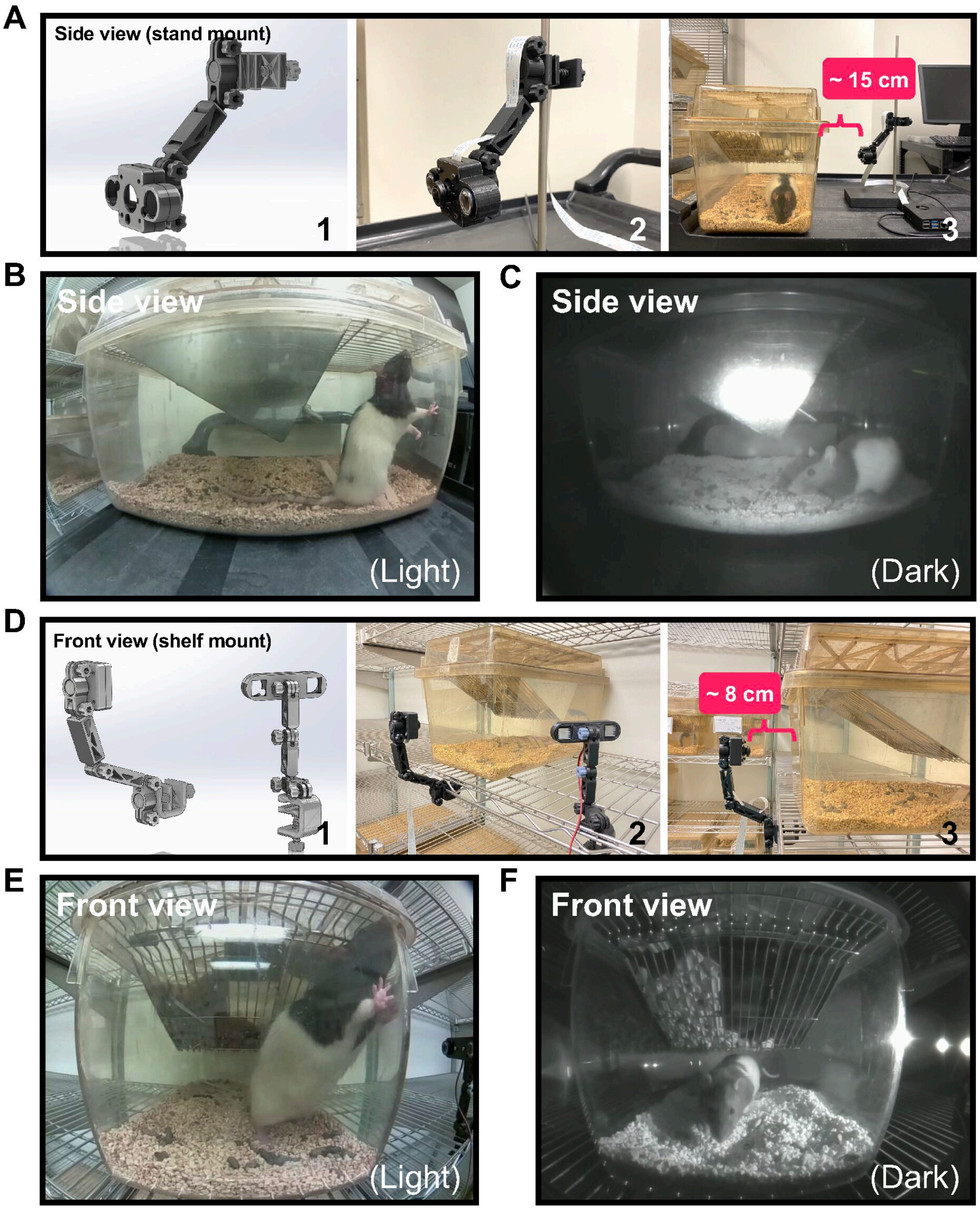
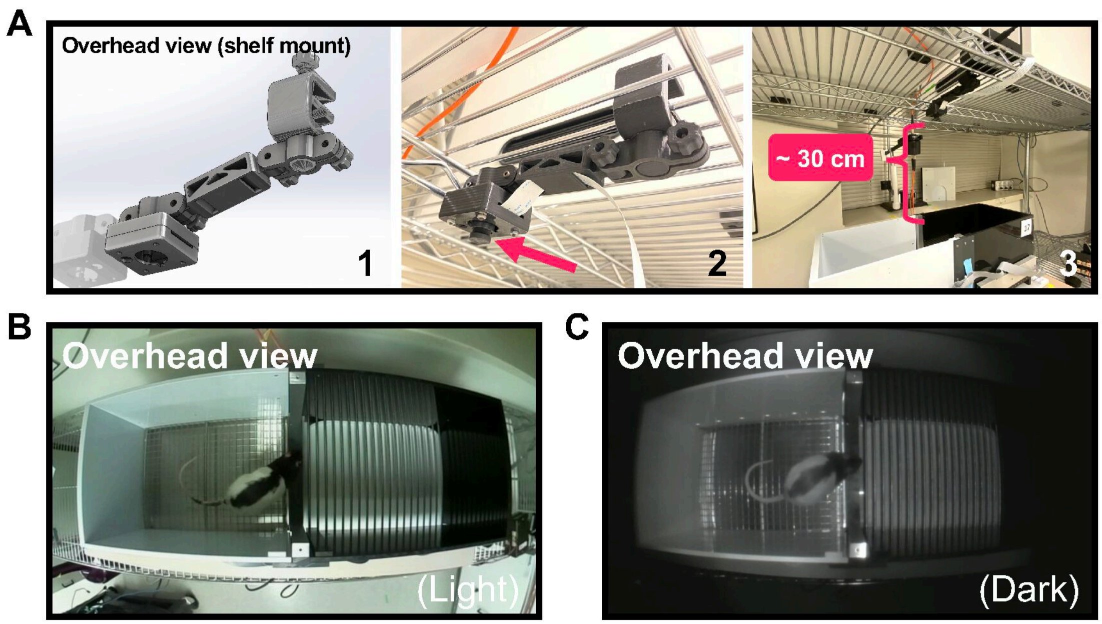
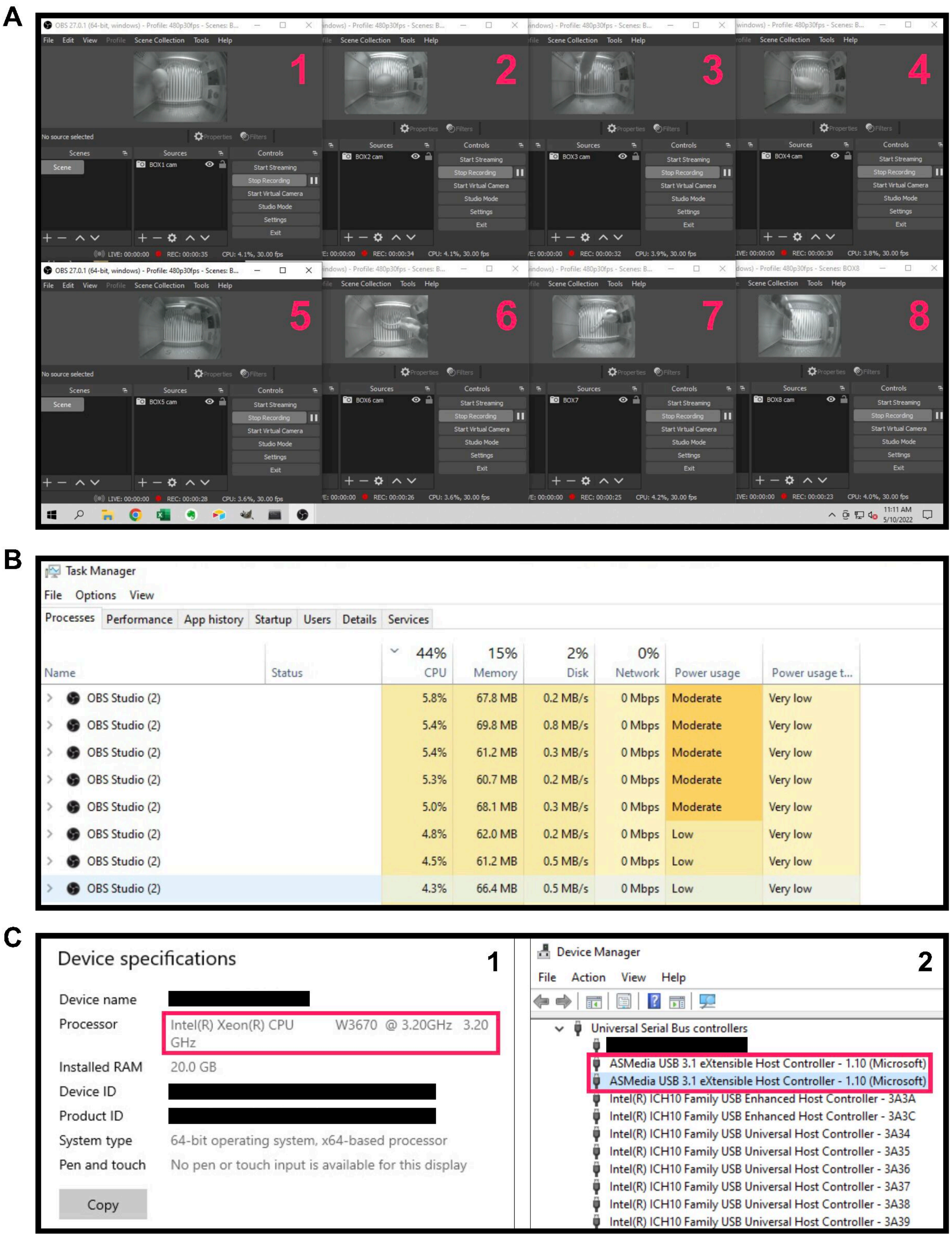



A
Overhead view (post mount)
1
2
< 1 cm
3
B
Overhead view (roof mount)
1
2
< 1 cm
3
C
Side view (post mount)
1
2
~ 5 cm
3
D
Side view (wall mount)
1
2
~ 5 cm
3
E
Overhead view
(Dark)
F
Side view
(Dark)


A
Side view (stand mount)
1
2
~ 15 cm
3
B
Side view
(Light)
C
Side view
(Dark)
D
Front view (shelf mount)
1
2
~ 8 cm
3
E
Front view
(Light)
F
Front view
(Dark)


A
Overhead view (shelf mount)
1
2
~ 30 cm
3
B
Overhead view
(Light)
C
Overhead view
(Dark)


A
1
2
3
4
5
6
7
8
B
Task Manager
File Options View
Processes Performance App history Startup Users Details Services
Name Status 44% CPU 15% Memory 2% Disk 0% Network Power usage Power usage t...
OBS Studio (2) 5.8% 67.8 MB 0.2 MB/s 0 Mbps Moderate Very low
OBS Studio (2) 5.4% 69.8 MB 0.8 MB/s 0 Mbps Moderate Very low
OBS Studio (2) 5.4% 61.2 MB 0.3 MB/s 0 Mbps Moderate Very low
OBS Studio (2) 5.3% 60.7 MB 0.2 MB/s 0 Mbps Moderate Very low
OBS Studio (2) 5.0% 68.1 MB 0.3 MB/s 0 Mbps Moderate Very low
OBS Studio (2) 4.8% 62.0 MB 0.2 MB/s 0 Mbps Low Very low
OBS Studio (2) 4.5% 61.2 MB 0.5 MB/s 0 Mbps Low Very low
OBS Studio (2) 4.3% 66.4 MB 0.5 MB/s 0 Mbps Low Very low
C
Device specifications
1
Device name [redacted]
Processor Intel(R) Xeon(R) CPU W3670 @ 3.20GHz 3.20 GHz
Installed RAM 20.0 GB
Device ID [redacted]
Product ID [redacted]
System type 64-bit operating system, x64-based processor
Pen and touch No pen or touch input is available for this display
Copy
Device Manager
2
File Action View Help
Universal Serial Bus controllers
[redacted]
ASMedia USB 3.1 eXtensible Host Controller - 1.10 (Microsoft)
ASMedia USB 3.1 eXtensible Host Controller - 1.10 (Microsoft)
Intel(R) ICH10 Family USB Enhanced Host Controller - 3A3A
Intel(R) ICH10 Family USB Enhanced Host Controller - 3A3C
Intel(R) ICH10 Family USB Universal Host Controller - 3A34
Intel(R) ICH10 Family USB Universal Host Controller - 3A35
Intel(R) ICH10 Family USB Universal Host Controller - 3A36
Intel(R) ICH10 Family USB Universal Host Controller - 3A37
Intel(R) ICH10 Family USB Universal Host Controller - 3A38
Intel(R) ICH10 Family USB Universal Host Controller - 3A39

Product	Vendor	Catalog Number	Price	Comment
RASPBERRY PI BOARD:				
Raspberry Pi 4 Model B (1/2/4/8GB)	anywhere	n/a	$35 - 75	Can be purchased as a kit with accessories at a higher cost
CAMERA MODULE:				
Arducam Wide Angle Day-Night Vision for Raspberry Pi Camera (FOV: 170° [D] x 140° [H])	UCTRONICS https://bit.ly/39PKPH8	B003507	$32.99	
ACCESSORIES:				
MicroSD Card (16GB)	PiShop.us https://bit.ly/38Nc45c	936	$7.95	Any microSD card with a minimum storage space of 64MB would work
Raspberry Pi 4 Compatible Heavy-Duty Aluminum Alloy Case with Pre-installed Ready to Connect Fan	Vilros https://bit.ly/3GnbeIQ	VILP015	$14.99	Any case with camera ribbon cable and GPIO access would work; Metal cases with pre-installed cooling fan would be preferred to prevent overheating of the Raspberry Pi board
Standard USB to USB-C Cable (6ft)	Vilros https://bit.ly/3alpw0C	VILP103	$4.99	Any USB-C type cable capable of data transfering
Jumper Cable Pin Header Connector Housing Assortment Kit	Amazon https://amzn.to/3z3Z0mu	B077X8XV2J	$12.98	For wiring the IR LED boards to use them independently of the camera board
Ring Terminal, Non Insulated, 22-16 Wire Size, #4 Stud Size	Amazon https://amzn.to/3LKqUH9	B005GDFMSG	$11.23	For wiring the IR LED boards to use them independently of the camera board
Red Black 2 Pin Wires 22 AWG (100ft)	Amazon https://amzn.to/3wJhnvN	B0793N3WZZ	$18.99	For wiring the IR LED boards to use them independently of the camera board
Heat Shrink Tubing Kit	Jameco https://bit.ly/3NxH5bX	2095963	$17.95	For wiring the IR LED boards to use them independently of the camera board
Crimping Tool Set	Amazon https://amzn.to/38Fy1mR	B0045CUMLQ	$66.74	For wiring the IR LED boards to use them independently of the camera board
Wire Stripper	Grainger https://bit.ly/3MOaMps	1XFZ6	$34.25	For wiring the IR LED boards to use them independently of the camera board
4-Port USB PCIe Card	CDW https://bit.ly/3aoX6Tz	6409687	$114.99	For expanding the total USB bandwidth of the host computer during multi-camera recording

STL file	Picture
CAMERA CASE:	
Pi_Cam_Case_Top	
Pi_Cam_Case_Bottom_w_Rod	
Pi_Cam_w_LED_Case_Top	
Pi_Cam_w_LED_Case_Bottom_w_Rod	
CAMERA MOUNT TOOLS:	
Pi_Cam_Arm_7CM_M2F	
Pi_Cam_Arm_7CM_M2M_90D	
Pi_Cam_Arm_7CM_M2M_180D	
Pi_Cam_Ring_90D	
Pi_Cam_Ring_180D	
Pi_Cam_Rod_Base	

STL file	Picture
IR LED CASE:	
Pi_LED_Case_Top	
Pi_LED_Case_Bottom_w_Mount	
IR LED MOUNT TOOLS:	
Pi_LED_Arm_5CM_M2F	
Pi_LED_Base	
Pi_LED_Ring_180D	
ACCESSORIES:	
M2.5_Nut_Knob	
G-Clamp_Clamp	
G-Clamp_Screw	
G-Clamp_Press	

Camera	Lighting condition	Recording length (s)	Video file length (s)	Target FPS	True FPS	Total dropped frames	Dropped frames per min
Commercial	Red house light	300.56 ± 6.08	124.77 ± 2.52	20	8.30 ± 0.00	3515.00 ± 71.25	702.00 ± 0.00
Pi USB cam	Red house light	300.60 ± 6.09	300.54 ± 6.09	20	20.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Commercial	White house light	311.05 ± 4.39	179.57 ± 17.43	20	11.57 ± 1.16	2628.60 ± 377.60	505.60 ± 69.40
Pi USB cam	White house light	311.02 ± 4.41	310.96 ± 4.40	20	20.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Camera	Lighting condition	Recording length (s)	Target FPS	True FPS	Total duplicate frames	Duplicate frames per min
Commercial	Red house light	301.29 ± 3.81	30	8.28 ± 0.01	6545.20 ± 83.92	1303.40 ± 0.24
Pi USB cam	Red house light	301.18 ± 4.09	30	30.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Commercial	White house light	300.37 ± 3.77	30	8.33 ± 0.02	6509.00 ± 79.33	1300.20 ± 1.20
Pi USB cam	White house light	300.65 ± 3.47	30	30.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00