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Microsco-pi: a novel and inexpensive way of merging biology and IT

Harry R. Kent and Jonathan P. Bacon

ABSTRACT It is well known that schools and colleges often have budget limitations that can hamper the effectiveness of practical education. This article looks at how cheap, off-the-shelf components can be used to produce a simple DIY digital microscope, and how this provides novel opportunities to integrate biology, physics, design technology and computer science in a fun and hands-on way.

Opening comments from Jonathan Bacon

I am convener of a first-year module at the University of Sussex on cell biology that is compulsory for most of our life sciences students. Every year I am surprised by the number of students studying biology, ecology and other life sciences courses who have never seen anything alive (and moving) under the microscope, yet have chosen these subjects at degree level.

Because of this, for the last few years I have offered a final-year project in which third-year students who are thinking about becoming secondary school science teachers spend one term studying microscopic animals in depth, with the aim of developing new practical experiments and demonstrations to accompany GCSE and A-level biology teaching. These students also get the chance to go into local secondary schools and lead practical sessions about hunting for living microscopic animals in moss and pond water.

Opening comments from Harry Kent

I was one of these final-year project students in 2014 and I quickly became fascinated by tardigrades, microscopic extremophiles that live in a variety of environments. I found it very hard to get a clear and stable image of the animals under the microscope using my mobile phone camera, so I tried to find some method of obtaining good images. While doing this, I came across an 'Instructable' guide from a member called Yoshinok (see *Websites*) that detailed how to produce a cheap DIY microscope stand using a smartphone camera to grab images. I built one to use as part of the teaching section of the project. However, it soon became clear that this tool could be improved upon to produce a digital microscope that might possibly become an integral part of my investigation into tardigrade locomotion.

After the final-year project had ended, we received some funding from the Technology Enhanced Learning Innovation Scheme at the University of Sussex to develop the digital microscope further. This article describes how we modified the initial design, lists the equipment required (Box 1) to reproduce one to use in schools and colleges for a range of different practical activities, and suggests further steps that could enhance the use of the microscope as a cross-curricular teaching tool.

We produced an online survey and found that only about a third of the 101 respondents recalled ever looking at living organisms under a

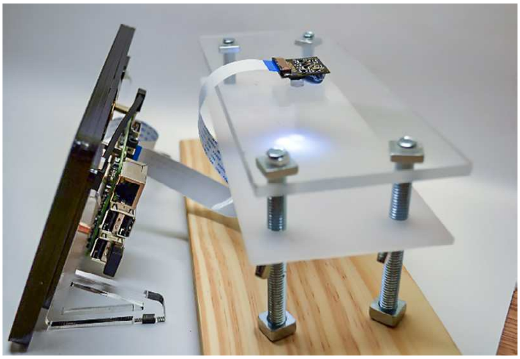

Figure 1 The entire 'Picroscope' assembly, with the stand on the right and the 7 inch touchscreen monitor on the left

Box 1 Equipment list

- wood;
- clear Perspex (200 × 80 × 5 mm);
- opaque Perspex (200 × 80 × 3 mm);
- four M8 roofing bolts 100 mm long;
- eight M8 nuts;
- four M8 wing nuts;
- short torch (approximately 4.5 cm in length);
- power drill;
- drill bit (8 mm);
- Forstner bit;
- Raspberry Pi 2 Model B;
- Raspberry Pi NOIR camera module;
- 7 inch Raspberry Touch Screen (or a monitor and the cable to connect it up);
- power cable;
- microSD card (8 GB minimum);
- Raspberry Pi wifi USB dongle;
- tealight candles;
- glass slides and coverslips;
- slide tool;
- USB keyboard and mouse;
- two laser pointers (or laser lenses);
- nail file;
- tweezers;
- can of compressed air;
- Blu-tack or hot-glue gun.

microscope. We wondered whether costs and staff time were key reasons for the lack of microscopy practical work in schools. SCORE (2013) released a report based on their survey of secondary schools which found that about 70% of schools felt they were not adequately equipped to teach science lessons effectively. One point highlighted was the lack of sufficient numbers of light and digital microscopes in classrooms; we address this issue in the following pages.

Many suitable components might be available from old phones or old computers. Some components will need to be purchased, but they are easily available from chain DIY stores, high street shops or well-known online outlets. The Raspberry Pi Foundation itself is a charity, and also operates its own store.

This article comprises three parts: setting up the Raspberry Pi; building the microscope; and uses and extension. The entire set-up could easily be built in an afternoon, or spread across a few weeks in lessons or as an after-school activity.

Part 1: Setting up the Raspberry Pi

The Raspberry Pi is a small credit-card-sized computer, used largely for education purposes. To date, seven models have been released (the A, B, A+, B+, 2, 3 and Zero) and over 7 million units have been sold worldwide (Code Club Blog, 2015). One of the key aspects of the Pi is the price, with the cheapest (the Zero) costing only £4. The most powerful, the Pi 3, costs around £30. They are very useful tools for teaching students computer science, and many companies have got involved. For example, Google donated 15 000 units to UK schools (Raspberry Pi Foundation, 2013). Raspberry Pis have been used in all sorts of research; the most recent and high profile example is the ‘Astro Pi’ project (astro-pi.org), which has seen British astronaut Major Tim Peake take two Raspberry Pi computers onto the International Space Station to run programs that school students have developed as part of a recent nationwide competition.

It is important to make sure that everything is set up and plugged in *before* turning the power on.

We used the Raspbian operating system, the officially supported variant of Linux. Raspbian is freely available from the Raspberry Pi Foundation’s Downloads page, or you can purchase a card with it pre-installed. Follow the online instructions to get the software onto the microSD card and insert this into the underside of the Pi 2. We used the most recent version (Raspbian Jessie, November 2015) for this article; older versions should work too, but the steps involved will be slightly different.

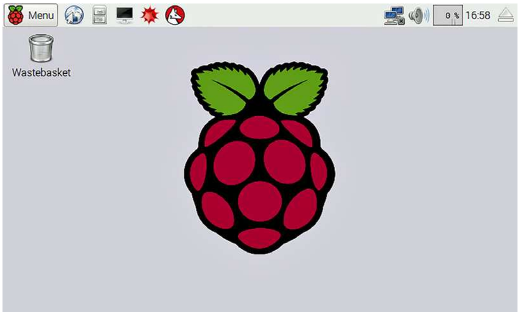

Figure 2 The Desktop of the Raspberry Pi; the newest version of Raspbian boots staight to desktop, as opposed to a login screen in the older versions

Figure 3 The menu bar found at the top of the desktop

Then, plug in your USB peripherals (mouse, keyboard, wifi dongle, etc.) and your HDMI lead. The camera is inserted into the CSI port (labelled as ‘Camera’ on the board). Finally, plug in your microUSB power cable and turn the power on. You will be guided through the installation process, which should boot straight into the Desktop, and will look something like Figure 2.

If you are using the 7 inch touchscreen or HDMIPi monitors, you will need to follow the instructions available from your manufacturer to set up and connect your monitor. We recommend you get the Raspberry Pi set up using a standard monitor and then, once you have a feel for it, hook up your purpose-built display.

Now, you should have a computer that looks somewhat similar to ones you are used to. The menu bar can be found along the top (Figure 3), along with some important program shortcuts. From left to right, these are the dropdown menu, the internet browser, the file manager, the terminal window, *Mathematica* and *Wolfram*. On the right-hand side of the screen, you will find the internet connection menu, the volume control, an indication of how intensely the Pi is operating (expressed as a percentage) and the time. To the far right is an eject symbol for the safe removal of USB sticks and external media.

To check whether your camera is working, click on the terminal icon and a small black box should appear. This is the terminal, where you will enter code to control the camera. Type

```
raspistill -t 0
```

This should bring up a live preview of whatever the camera is imaging. A list of commands for the camera can be found in Box 2.

One great advantage of using a Raspberry Pi as the basis for the digital microscope is that it can run programs locally, without the need for an additional computer. There are all sorts of programs that can be run on the Raspberry Pi, but Box 3 shows some of those that we recommend for a school setting, and lots of information can be found by looking at the *Websites* section below.

Box 2 Camera commands

-
  ```
  raspistill -o insertyourimagename.jpg
  ```
  - This is the command you use to save an image with the filename you want to choose. –o is the output argument, and is used to name files. It will save by default to your home folder.

-
  ```
  raspistill -o /home/SSR/Figure1.jpg
  ```
  - This command saves Figure1 into a folder called SSR which is found in the home directory. You can change where files are saved this way, but be careful to check that the folder you want to save an image to has already been created.

-
  ```
  raspistill -t 600000 -tl 10000 -o timelapse_%04d.jpg
  ```
  - This command produces a time lapse. The –t arguments denote the entire time you want to produce a timelapse over (in milliseconds), -tl defines the time period between photos (in milliseconds) and –o will give the image a filename. By using _%04d in the filename, the Pi will give each image captured a four-digit identifier, which increments by 1 over the time lapse.
  - In this example, a time lapse will be created with pictures captured every 10 seconds for a ten-minute period. The saved files are called ‘timelapse_0001.jpg’, ‘timelapse_0002.jpg’, etc., up to ‘timelapse_0060.jpg’.
  - If you are planning to analyse these sequences, we recommend that you copy and paste each time lapse into its own folder to make later analysis easier.

-
  ```
  raspivid -t 10000 -o moviename.h264
  ```
  - raspivid is the command used to instruct the Pi to capture a movie. The –t defines the time in milliseconds for which to capture footage, and –o is used to choose a filename. The movies are in .h264 format, which can be played back on a Linux, Mac or Windows PC using the free *VLC* media. player.

Dropbox Uploader is a program by Andrea Fabrizi that allows the user to send pictures captured on the Pi to a *Dropbox* account, which can then be accessed on any other computer or tablet with a web browser and access to the internet. This is great for sharing work, or for getting images for reports and presentations. The uploader can save new files in the *Dropbox*

Box 3 Programs recommended for the Pi

- *Dropbox Uploader* (github.com/andreafabrizi/Dropbox-Uploader)
- *ImageJ* (Schneider, Rasband and Eliceiri, 2012; imagej.nih.gov/ij)
- *MTrackJ* (Meijering, Dzyubachyk and Smal, 2012; imagescience.org/meijering/software/mtrackj)

account and also send entire folders across with a simple command. Equally, you can send images from another source, such as a mobile phone, to the Pi itself for analysis.

ImageJ is a free image analysis software package that is often used for scientific research. Installing *ImageJ* is simply a matter of typing the words ‘`sudo apt-get install imagej`’ into the terminal window and then typing ‘`Y`’ when prompted. This will install *ImageJ* onto your device. You will find it under ‘Menu’|’Graphics’. The increased processing power of the Pi 2 allows *ImageJ* to run smoothly and to load up a large number of image files. However, this is quite an intensive task that even on high-specification computers can take several minutes, so patience during image loading is required.

To import an image sequence, simply go to ‘File’|‘Import Image Sequence’ and then select the folder in which the images are kept.

MTrackJ is an *ImageJ* plug-in that works alongside *ImageJ* to provide the ability to track changes in images over time. It has been used, for example, to count cells and to look at leg movement coordination in a variety of animals, and we used this plug-in to monitor tardigrade locomotion in various conditions. *MTrackJ* allows the user to highlight one point on a frame. Then, you can load the next frame, and highlight the new position of the thing of interest (e.g. a tardigrade). You then iterate through the entire sequence of images, building up a motion path of the target. You can then save the path as a movie or you can output information about the distance moved.

Part 2: Building the microscope

Now that you have a working computer that is capable of capturing, saving and analysing images, you can start to assemble the microscope. A step-by-step guide for a small-size model is given in Box 4.

Because of the flexibility of the design, you can adapt it to your needs. As this project focused on examining organisms in a small, enclosed area on a slide, we made a smaller base. If you want to examine a larger area, for example a Petri dish, you can make the specimen stage larger so you can move your sample around more freely. The basic mechanism is the same: wing nuts are used to move the specimen stage closer to, or further away from, the camera, essentially acting like a focus knob on a traditional microscope.

Part 3: Uses and extension

As previously mentioned, the Raspberry Pi has been used in all sorts of research projects around the world. Their low cost and power requirements, great flexibility and ease of use make them ideal tools. For example, they have been used to study penguins in the Arctic, to control camera traps to study biodiversity under water, for educational high-altitude ballooning and for monitoring air quality.

This equipment was used at the University of Sussex to study the behaviour of tardigrades when exposed to various environmental conditions. Tardigrades are microscopic members of the Ecdysozoa, and are some of the toughest creatures known. Discovered in 1773 by Goeze, they were initially known as small water bears (Figure 5). They are able to enter an ametabolic state, known as the ‘tun’ form, in which they can survive pretty much any environmental extreme, including pressure, radiation, temperature, dehydration and even the vacuum of space. For a review of tardigrades’ ability to resist environmental change, see Møbjerg *et al.* (2011). However, one of the most incredible things about tardigrades is that they can be found practically anywhere, from the Arctic to tropical regions, and even in the moss on your school’s grounds.

Owing to their inherent cuteness and near indestructability, tardigrades are hugely popular with students. Their alien-like qualities make them a really engaging subject, and they lend themselves readily to cross-curricular lesson planning.

As well as finding different, unidentified species in moss, we focused on one species, *Dactylobiotus (Macrobiotus) dispar*, purchased from Sciento, Manchester. A very handy characteristic of *D. dispar* is their transparent nature, which means that the contents of their gut

Box 4 Microscope construction

Firstly, you need to get some lenses. These can be bought (see AixiZ in the *Websites* list) or you can get some by taking laser pens apart. Carefully open up the laser pen using pliers (first making sure that no battery is in the pen and taking care not to get cut on the sharp metal) and remove the lens. It may be in a plastic holder; if possible, remove the lens, or use a hacksaw to cut the plastic down, and then file for a smooth finish. Use the can of compressed air to clean the lenses. We found that you can stack two lenses next to each other to get a good level of magnification (Figure 4A–D), but your lenses may vary.

Take the piece of wood and mark four drill points (which will depend on the size of your stages). This will act as your base. Drill holes through these points and then, once you know the roofing bolts will fit through, turn the base over and countersink the holes using the Forstner bit, allowing the base to sit flush on the work surface. An alternative is to use a spade drill to make suitable holes to inset each screw head before drilling the holes through the wood.

Then, take the Perspex and mark drill points 15 mm from the long edge and 20 mm away from the short edge. The opaque Perspex will act as the specimen stage and is designed to distribute the incident light across the surface (Figure 4E) as opposed to bleaching out the image by beaming directly into the camera. The clear Perspex will act as the camera stage. Once you are happy that these stages will fit onto the roofing bolts and the specimen stage is able to slide up and down freely on the bolts, you are ready for the next step. You may want to use a small file to increase the diameter of the holes, depending on how much freedom of movement your set-up has.

Take the camera stage and drill a hole in the centre. This hole has to be almost the exact same diameter as your lenses, so it will depend on your own set-up. Once this hole has been drilled, simply place the lenses in it. If they do not sit correctly by themselves, either use a file to open the hole a bit more, or use Blu-tack or a hot-glue gun to fix the lenses in place.

Finally, once you are happy that it all fits together, take the stand apart and mark a point directly below the lens with a pencil. This is where your light source will be located. Using the Forstner bit that is a similar diameter to the base of the torch, drill a shallow hole in the upper surface of the wood layer to hold the light source. If you do not have a small torch that will fit, a smartphone's flashlight app also produces a good quality light.

Using the bolts, nuts and wing nuts, assemble the stand as shown in Figure 4E. As it is, this set-up can be used in a classroom or outdoor setting with camera phones to look at microscopic detail. The wing nuts allow you to move the specimen stage relative to the camera stage to bring the image into focus.

To fit the Pi in place, simply line up the camera module with the microscope's lens, and fix with Blu-tack. You could drill some holes to line up with the mounting holes present on the camera board and screw the camera board into place. To position a phone or tablet, open the camera app and line the phone/tablet camera up with the lens.

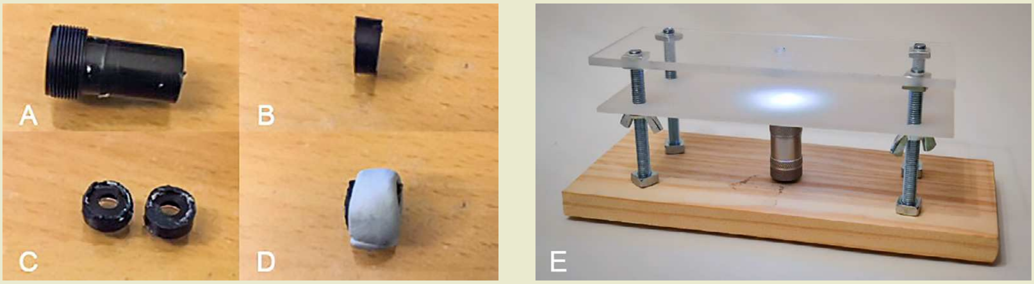

Figure 4 (A) How the lens is mounted in a laser pen; (B) using a hacksaw, you can remove excess plastic and file it down; (C) repeat the process to get a second lens; (D) you can use Blu-tack to hold two lenses together; (E) the complete, assembled DIY microscope stand; without the Pi in place, this can be used with a camera phone or tablet to produce high levels of magnification

Figure 5 This is the first illustration of a tardigrade by Goeze: a 'Kleiner Wasserbär' (Bonnet and Goeze, 1773)

are very visible if they have been grazing on moss and stand out against the background; this acts as an ideal reference point for image tracking.

The investigation into tardigrades was partly inspired by an article by Shcherbakov *et al.* (2010), who used custom software and expensive equipment to plot tardigrade locomotion. Our DIY version relies on things that you can easily obtain. The set-up is as follows. A layer of wax from tealight candles is poured across a glass slide. To this, a small Perspex sheet with a 5 mm hole drilled through the centre is added. This set-up can be held over a gentle flame to remelt the wax to fix the Perspex and thus to obtain a smooth surface within the observation arena into which the tardigrades are placed.

Then, using the commands from Box 2, a time lapse recording was produced. We took one picture every ten seconds over a ten-minute period, but this can be adjusted to meet your needs. These images can then be loaded into *ImageJ*, and then *MtrackJ* can be used to plot the path of the tardigrade(s) (Figure 6).

You can vary the conditions of the experiment (e.g. light/dark, temperature) to produce an investigation for the students. For example, Figure 6 shows the same tardigrade in the same arena at different temperatures.

Just by taking tardigrades as the focus of a multidisciplinary lesson plan, you can develop a whole project that crosses between biology, physics and chemistry and incorporates some engineering and computer science. We believe that this approach would help students see that the STEM subjects are not separate modules but that research relies on subjects working together to find the solutions to the big issues.

The Pi comes pre-installed with several different development environments to allow students of all ages to learn about coding. A simple drag-and-drop system called Scratch is often used with younger students. There is also Python, a great coding language for beginners. It also has BlueJ installed, which is the Java coding environment that is used to teach programming modules to first-year computer science students at the University of Sussex. The Raspberry Pi has all the software your students will need to start coding. As more GCSE and A-level teaching becomes focused on the principles of coding,

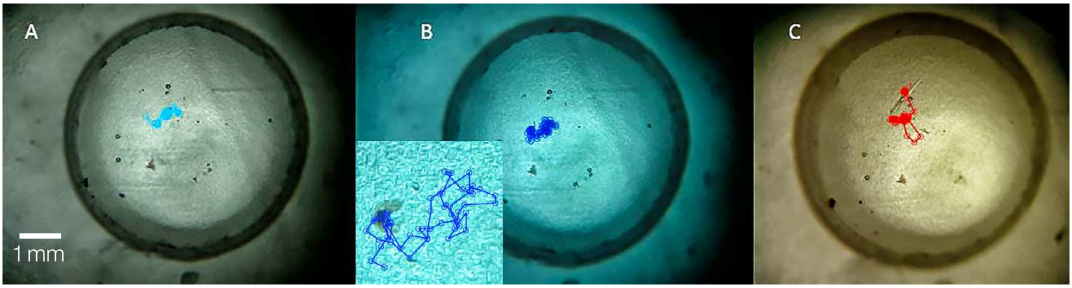

Figure 6 An individual tardigrade was monitored at three different temperatures over a ten-minute period with an image taken every ten seconds. The path was added using *MTrackJ*. The tardigrade was left to acclimatise for ten minutes in each condition before being observed at (A) 8 °C, (B) 20 °C and (C) 32 °C. This tardigrade showed a maximum speed of approximately 3 mm min^{-1} (compared with a body length of roughly 0.25 mm) at 32 °C. Image (B) shows an expanded view of the motion path and the tardigrade is clearly visible as the grey outline on the left.

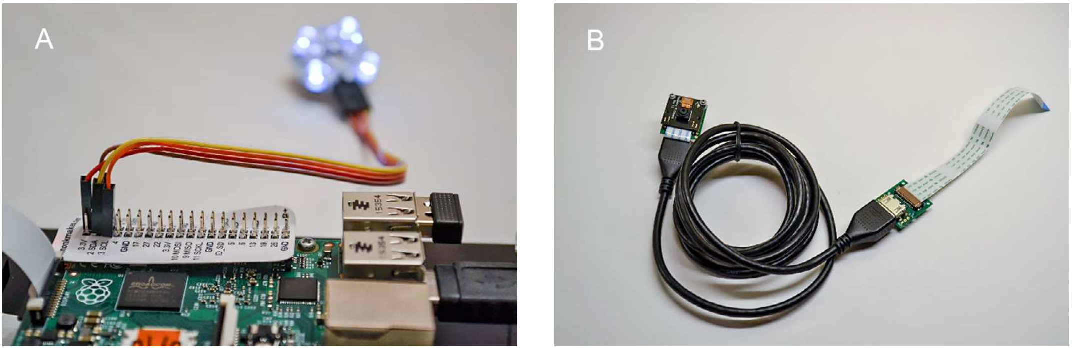

Figure 7 (A) BrightPi set-up, with white LEDs turned on; (B) HDMI camera extension kit

the Pi will become an even greater tool, and its use in different subjects is something that more conventional school equipment cannot easily replicate.

Extension

As has already been stated, one of the best things about this set-up is its flexibility. There are many ways that you could expand upon the basic tool built in this project.

You could use a USB power bank to provide power to the microscope to allow it to be used away from a mains supply. A good-quality power bank that outputs at least 2 A should allow the Pi and some screens (such as the 7 inch touchscreen) to be used on the go.

We have subsequently replaced the stubby torch with a BrightPi, a kit produced by Pi Supply, which consists of four bright white LEDs and eight infrared LEDs (Figure 7A). This allows the light levels to be controlled by the user using some commands typed into the terminal window. The BrightPi requires soldering, so students can learn a new skill in the process.

We have also attached an HDMI camera extender (Figure 7B). This allows a standard HDMI cable to be used as the link between the camera and the Pi. The standard cables between the Pi and the camera are about 20 cm long, but 5 m HDMI cables can be readily purchased. Using the infrared camera on the microscope and an infrared light source, the set-up can be placed in a dark environment to monitor the effects of light and dark on animal behaviour, and the long camera lead allows for the user to observe and interact with the touchscreen display in normal working illumination.

We are also currently working on motorising the specimen stage. We are aiming to use an Arduino (another microcomputer) and stepper motors (salvaged from scrap CD and DVD drives) to control the position of the stage in the *x* and *y* planes. This will enable the DIY microscope to be used with greater accuracy and also gives students even more opportunities to learn about basic electronics and coding.

Conclusion

The set-up provides a great starting point for a really easy-to-use mixture of biology and computer science, as well as design technology and also some basic physics. The fact that students can get a chance to see how different subjects can support each other in a classroom setting is really exciting, as many students often get taught subjects in isolation, and the ability to link ideas across disciplines is a skill that is very useful to acquire when young.

The DIY microscope is ideal for use as a long-term project, such as an after-school club, or for use in short in-class practical demonstrations. Once you are comfortable with the Pi, there are many other practical tools that can be produced very cheaply (light gates, thermometers, soil moisture monitors and much more). We hope that this guide will encourage school staff to see that they can produce their own equipment on site at a fraction of the cost of purchasing and maintaining traditional equipment, and get students involved in the production, evaluation and improvement stages of the build.

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