# BYOE: Determination of Diffusivity via Time-lapse Imaging with a 3D-Printed Spectrometer and a Raspberry PI

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## Abstract

Mastering the concepts of diffusion is crucial for engineering students as it is a vital process of mass transport in both physical and natural sciences. However, deciphering this phenomenon while connecting the theoretical models developed by Fick to real-world data collected in a lab can be challenging for students. Measuring the diffusion process accurately enough to calculate diffusivities often requires cost-prohibitive instrumentation for many teaching lab applications. Other methods require complicated preparation and planning, which obliges the students to spend most of their time troubleshooting the setup rather than on the primary student learning outcome.

This “Bring Your Own Experiment” (BYOE) paper presents a simple, low-cost experiment developed to overcome these challenges and help students understand diffusion through experimental design, visualization of diffusion profiles, and mathematical modeling. The experimental setup consists of a 3-D printed spectrometer, Raspberry Pi Zero W, Raspberry Pi camera, and custom in-house written time-lapse Thonny code for Raspberry PI and semi-micro cuvettes. Students are asked to examine the relationship between agar hydrogels' properties and a food dye's diffusion. The equipment takes an image of a cuvette at set time points. Image J is used to analyze the images taken by the camera. A calibration curve relating the RBG color saturation of the food dye to the concentration is created. Once the calibration is completed, timelapse diffusion experiments begin. Students must decide how long to run each experiment, how often to image the cuvette, and the range of agar weight percentages to test. Cuvettes filled with 1 mL of agar hydrogel will be loaded with 1 mL of a high concentration of food dye on top. Using the calibration data and the timelapse experiments, students then model their time-dependent concentration profiles and calculate the diffusion coefficient for each dye-agar system. The final product the students are asked to create is a report of their analysis to include sample images, plots of the concentration profiles with their modeled data, and a discussion of their result in which they should address the relationship between the varying agar weight percentages and the diffusion of the dye.

The ability of the instrument utilized in this BYOE to image a time-dependent 2-D colorimetric response has multiple applications, such as relating the effect of molecular weight and molecular shape to diffusion coefficients, enzyme kinetics with a colorimetric reaction, and thermodynamic experiments measuring changes in heating/cooling curves utilizing thermochromic dyes with the addition of a small piezoelectric device. The versatility of this low-maintenance, economical, innovative experimental apparatus can be used for innumerable applications across many engineering curriculums.


## Introduction

Our role as scientists in education is not only to further the understanding of the natural world in which we live and the connections between matter, energy, and life using the scientific method to develop hypotheses and theories based on results through observation and careful testing. But

also, to teach our students these skills. One of the most daunting tasks in engineering education is teaching our students how to apply mathematical models to interpret our observations that will accurately predict future outcomes [1], [2], [3], [4], [5], [6], [7], [8]. In most cases, if the phenomenon under observation is on the human scale and occurs in our natural frame of reference, these models can be easily demonstrated, observed, evaluated, and implemented. For example, a middle school physical science class may use Newton's law of gravity and one-dimensional to calculate the time a ball to hit the floor as it dropped from different heights. Depending on the student's mastery of mathematics, some students may initially struggle with the computations involved with this phenomenon; most can accurately describe the mean of each variable in the equation and, in their own words, the relationship these variables have with one another [9].

Like the example above, countless experiments and demonstrations are done throughout science classrooms around the world that couple direct observations using our five senses with scientifically proven mathematical models that accurately describe the interactions between momentum, energy, and matter that occur on the human scale and can be directly observed with and manipulated as part the five-stage learning cycle that many students that have excellent math skill master with a modest amount of effort [10], [11], [12]. Unfortunately, as we begin to explore areas and interactions on a scale that makes it impossible for the students to observe or manipulate directly, their ability to master these concepts quickly diminishes. Often, this is attributed to the lack of mathematical readiness on the student's part. Still, even the best pre-service physics teachers struggle with mastering phenomena covered in special relativity, even portions governed by straightforward mathematical equations such as time dilation as described by the Lorentz factor and Einstein’s theory of special relativity [13]. Students are not suddenly incapable of performing the mathematical steps presented before them; instead, they lack understanding of the system and its connection to their experience, current knowledge, and the mathematical model [14]. When we dive into more abstract and complicated systems that are described in a text or those that can only be observed indirectly, such as modeling the diffusion breakthrough curve of a semi-infinite source into a finite well with all the appropriate boundary conditions and numerous variables, the situation is further exasperated. The real challenge lies in aiding students in creating an accurate mental image that allows them to connect the phenomenon and its mathematical construct with a clear understanding of each of the parameters that affect the resulting phenomena.

Over the last several decades, numerous approaches have been employed to improve students' ability to make these connections. Many include a spectacular visual aid, physical manipulatives, and demonstrations [15], [16]. Although these have been excellent approaches to help students generate a mental image of the system in question, often, these teaching aids are expensive or on a scale that does not directly correlate with the mathematical expressions, leaving the students to infer and build the complete mathematical picture themselves. Other approaches that have been employed include computer-generated models. While these can help students understand how a specific variable can affect the outcome of an experiment, they often need to be more simplified or more complicated to implement. What is presented here is a device that allows the student to interact directly with a system that not only creates a visual aid via time-lapse imaging but also

can be analyzed with known mathematical models for diffusion. Repeated experimental runs allow students to observe directly how initial concentration and time impact the system's diffusion profile.

The experiment focused on the students developing their skills in experimental design and expanding their understanding of the diffusion process. They are provided with the prebuilt and preprogrammed time-lapse spectrometer to reduce the distraction from the lesson's primary goals. They were allowed to develop their process to achieve the end goal by developing their calibration curve, experimental runs by varying the concentration of the diffusing dye, and their own appropriate choice of modeling to determine the diffusion coefficient of the green food dye.

**Fabrication of Device**

The spectrometer box comprises five 3-D printed parts, as shown in Figure 1, using white polylactic acid (PLA) filament. Before assembling the spectrometer box, six 5mm ~3.0 V white LEDs were inserted into the LED Bracket. The holes of the LED bracket were angled inward at ~18.2 degrees so that each LED right and left pairs' illumination would overlap at a distance of ~ 6cm, coinciding with the distance of the cuvette.


**Figure 1.** The cover of the spectrometer (A). The base of the spectrometer box with an integrated semi-micro cuvette holder (B). The back wall of the spectrometer with a slit to accommodate Raspberry Pi Camera ribbon cable and wires for LED power (C). The bracket is to hold six white LEDs (D). The mounting bracket for the Pi Camera (E).

Once the LEDs were placed in the bracket, they were wired in parallel to each other and series with a 50-ohm resistor. A read wire with a female jumper connector was added to the end of the resistor to facilitate the connection to the Raspberry Pi Zero W pinout 12 corresponding to GPIO18. A green wire with a female jumper connection was added to the cathode side of the LEDs to connect them to the Pi Zero W ground, pinout 39, as shown in Figure 2A. Once the

wiring was complete, the positive and negative leads for the LED assembly were threaded through the side ports of the camera holder and snapped into place. The Pi Camera was placed on the opposite side with the connector for the ribbon cable placed facing up, and everything was held in place with two M2 x 0.40mm by 6mm long pan head screws, Figure 2B. Once assembled, the ribbon for the Pi Camera was connected to the camera. To finish the spectrometer box, the back wall shown in Figure 1C was attached to the spectrometer box, Figure 1B using four 3M x 0.50mm by 5.5 mm long button head cap screws. The screw holes in the 3-D printed part were sized such that the screws cut their thread, and no nuts were needed to hold the part together. With the base of the spectrometer assembled, the wires for the LED/Camera assembly were threaded through the slit in the bottom of the back wall (part C), and the assembly was dropped in place. To finish the construction of the spectrometer, the ribbon cable and positive and negative leads were connected to the Raspberry Pi Zero W, see Figure 2C. The list of materials to complete the spectrometer is in Table 1. Once assembled, the Python code, shown in Appendix A, was created to record calibration, and the timelapse images for the diffusion experiments were loaded onto the Pi Zero W.

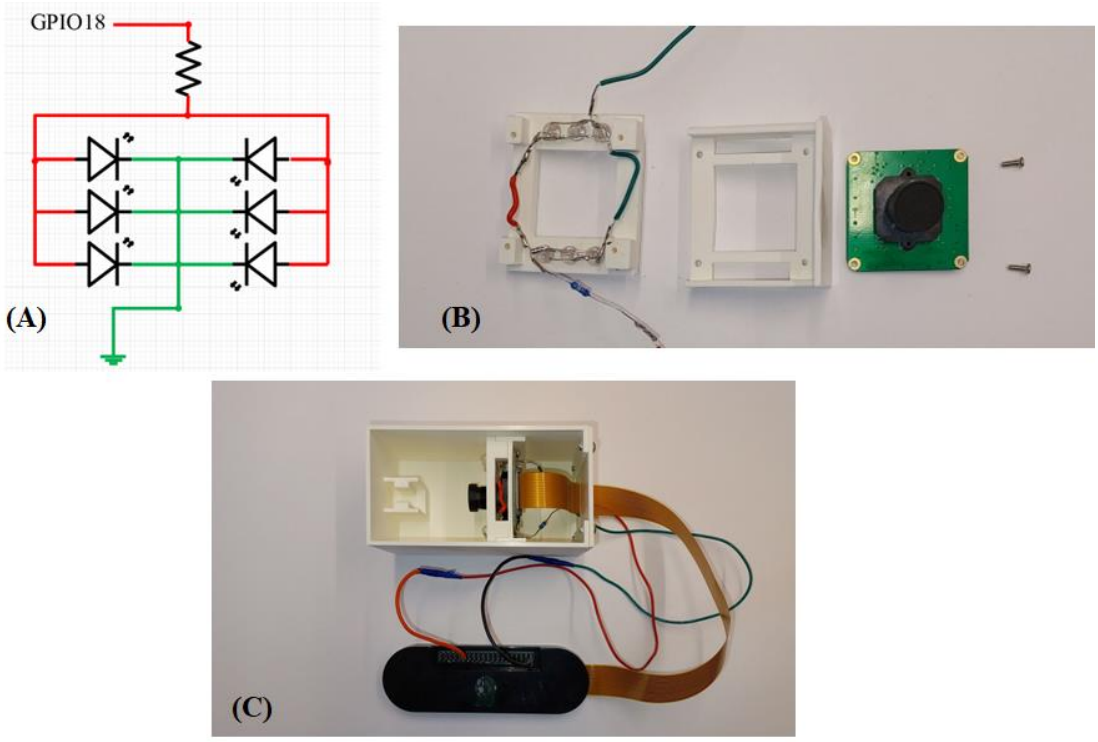


**Figure 2**. The circuit diagram for the LEDs and 50-ohm resistor (A). The assembly order of the LEDs, camera bracket, and Pi Camera (B). The final assembly of the spectrometer is shown above in (C)

**Table 1**. List of materials and peripherals

| Items for the spectrometer | Vendor | Part # | Unit price | qty |
|---|---|---|---|---|
| CanaKit Raspberry Pi Zero W | Amazon | N/A | $66.02 | 1 |
| Innomaker Raspberry Pi Camera | Amazon | N/A | $10.39 | 1 |
| 30 cm Ribbon flex cable for Pi Zero W (2pk) | Amazon | N/A | $8.95 | 1 |
| 250 pc 5mm Assorted LED | Amazon | N/A | $8.89 | 1 |
| USB-wired Keyboard and Mouse | Gov Connect | AP-BM530 – b3e | $12.71 | 1 |
| 4 port USB2.0 mini hub | Gov Connect | U222-004 | $6.78 | 1 |
| Acer 21.5 Lcd monitor | Gov Connect | KA220Q B | $72.31 | 1 |
| | | Total | $186.05 | |

Many of the other parts, such as wire, screws, and soldering supplies, were on hand but could be easily obtained at Digi key or McMaster Carr.

**User Interface**

The assembled device was given to each student group to run their time-lapse experiments along with directions on how to use the Raspberry Pi Time-lapse device. An example of these directions is shown in Appendix B. To run their experiments, students needed to create a calibration curve to relate the color intensity to the concentration of the dye. There was a separate program file that led the students through the process. First, the students created their calibration solutions of dye in water using good analytical techniques. The program displayed prompts in the window to provide instructions for the students. It first asked how many calibration solutions they created. The students then loaded a calibration solution into a clean cuvette, entered the concentration into the window, and an image was taken. The image file name displayed the entered concentration and saved it in a file folder of the student's choosing. This procedure was repeated until images of all calibration solutions were collected, and the team was ready to move on to the next step.

Before beginning the time-lapse diffusion experiments, each group of students needed to determine their experimental plan. The time-lapse program allows students to decide how long to run the trial and how often an image is taken. Due to the limited processing power of the Pi Zeros, it was recommended that students perform the image analysis on a second computer using ImageJ [17]. Students were allowed to determine their analysis approach; however, an acceptable approach for image analysis was given to the students and is provided in Appendix C.

**Implementation into Course**

Groups of three to four students in the third year biomedical engineering curriculum were presented with the experimental objectives of analyzing the relationship between agar hydrogel properties and the diffusion of a dye. They were provided the preprogrammed Raspberry Pi Zero W with a Pi camera with all the peripherals required to perform the diffusion experiment. Also, a supply of disposable semi-micro cuvettes, agar powder, and a stock solution of green food color dye with a concentration of ~250 μM, along with basic laboratory equipment, was available in the classroom. Students were expected to design an experiment to accomplish their experimental objectives. Some parameters they had to consider were the number of concentration points used

in their calibration curves, the range of agar percentages, and the duration of the experiment. For the time-lapse run, they needed to determine how long to run the experiment, the number of images to record, what concentrations of agar gels were used, and the number of replicates.

This laboratory course is one of the two required writing-intensive classes in the curriculum. There is an emphasis on both written reports and oral presentations. For this experiment, each team of students was expected to submit a proposal with their experimental design plan, present their results and conclusions to their classmates, and complete a closing report of these findings. This was conducted over five weeks, thus giving students a chance to reassess their original experimental plan and make any necessary adjustments. One of the first steps each team of students had to complete was to calibrate their experimental apparatus. Figure 3 below is an example of the calibration data measured by students.

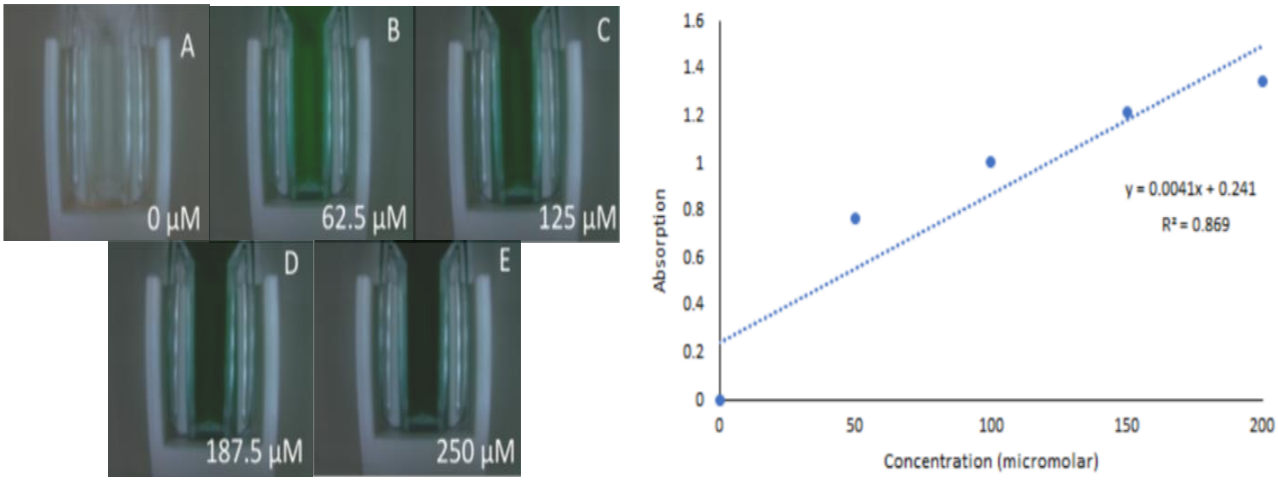


**Figure 3**. Example of the student calibration procedure. (Left) The device took images of varying concentrations of dye. (Right) Resulting in a trendline from the analysis of the images.

Each team generated concentration profiles to evaluate the relationship between the agar hydrogel's properties and the dye's diffusion. Figure 4 displays a plot of a team's key results. Students observed the dye's progression into their cuvette of agar hydrogel, which allowed them to conclude that an increase in the amount of agar present in the hydrogel decreases the rate of change for dye concentration in the gel. Progression of the dye into their cuvette of agar hydrogel. This was visible in their images taken over time and echoed in the analysis of the concentration profiles.

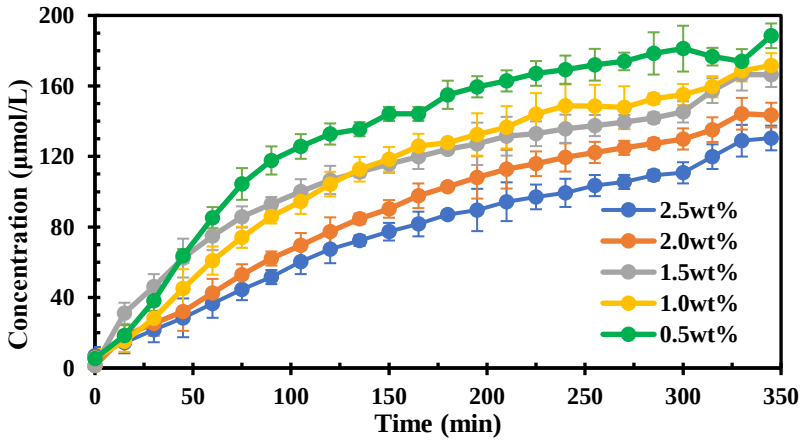


**Figure 4.** The plot shows the dye concentration over time for triplicate trials of five agar hydrogel weight percentages. The error bars display the standard deviation. This was a figure presented as critical results from a team of students.

Students were expected to calculate the diffusion coefficient for each agar weight percentage gel they tested. The system is assumed to be a semi-infinite media and obeys Fick's second law. Using the created calibration curve shown in Figure 3, they converted the pixel density of each image from the timelapse experiment into dye concentration. The initial condition of the concentration of one milliliter of dye at 250 micromolar was deposited on top of the agar hydrogel at time zero. Using the calibration curve to find the concentration at every point in the hydrogel every time an image was recorded, students could calculate a diffusivity for each weight percent hydrogel.

Interestingly, the concentration profiles created by the student, as shown in Figure 5, presented an excellent teaching moment. The question was posed: if the concentration at the interface (concentration values displayed in Figure 5 at position 0 mm) at time zero was assumed to be 250 micromolar, why does it decrease with increasing weight percent? The model they used to calculate diffusivity indicates that this concentration should be 250 micromolar for each run at the interface. Using the images and data, they recorded the hypothesis that increasing the weight percentage of the agar hydrogel imparted a partition coefficient, which was not part of their original analysis. Reviewing the images and data-rich analysis, the concept of a partition coefficient and how it will affect the boundary condition was evident to the students. In previous years, this concept was not addressed in this experiment due to the equipment's inability to measure the diffusion in as much detail as the time-lapse spectrometer presented here.

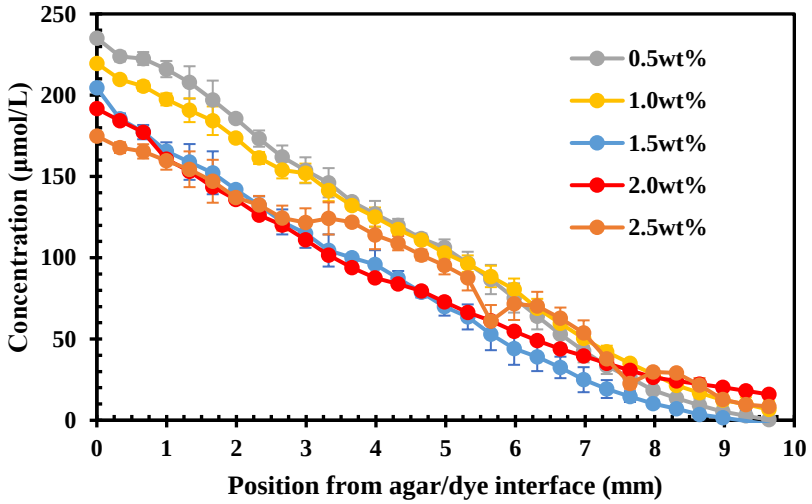


**Figure 5**. The plot shows the dye concentration in the agar hydrogel for triplicate trials of five agar hydrogel weight percentages. The error bars display the standard deviation. This was a figure presented as critical results from a team of students.

## Conclusions and Future Work

The implementation of this new diffusion experiment into the laboratory course went well. The equipment performed flawlessly. The students could interact with the interface and set their desired parameters. Each trial's data was available and analyzed with little trouble. Each student team was able to develop and conduct an appropriate experimental plan, analyze the data, and draw conclusions from it. We were also able to use mathematical models with the experimental data. The value of the learning objectives from this experiment greatly outweighs the capital cost.

The students have received the described device well, with countless possible applications. Current plans include modifying this device to incorporate a piezoelectric device and temperature sensor so that students can control the internal temperature of the spectrometer. This addition would allow the student to investigate the effects temperature may have on diffusion while increasing the versatility of the timelapse spectrometer. This would also allow the spectrometer to be utilized for kinetics lab experiments, both with and without enzymes, that provide a colorimetric response. There are also plans to 3-D print a camera holder capable of suspending the camera over a petri dish so the time-lapse experiments can be performed that allow students to investigate radial 2-dimensional diffusion from a point source. This arrangement, with careful planning of the stationary and mobile phases, could enable students to study more complex diffusional dynamics with direct applications to drug delivery. This arrangement, coupled with a heating source and various materials such as aluminum, steel, brass, and Pyrex strips coated in thermochromic dyes, should allow the students to create time-lapse heating and cooling curves.

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**Appendix A. Python code**

The Pi camera library is very versatile and includes extensive functions for image capture, video capture, and analysis. Although the default settings, such as the auto exposure and auto gains, were very good at capturing high-quality images, it was discovered that these functions would update for each image, making analyzing calibration and long time-lapse runs challenging. To overcome this challenge, preliminary code was written to measure the initial settings determined by the auto functions and lock these parameters for the whole calibration or time-lapse run. For this prototype, the Python code is broken into two separate applications. One is used to create images for the calibration curve to relate color intensity to dye concentrations. The Second is to run a timelapse capture sequence of the food dye diffusing through the agar gel samples. Since there are two different applications, the initial code is identical and reports the settings of the gains so that the user can determine that they are equivalent in both cases.

*Initial code for PiCamera*

```
# Loads the necessary packages
from picamera import PiCamera
import time
from tkinter.filedialog import askdirectory
import os.path
import RPi.GPIO as GPIO
import sys


#setting the GPIO pin 18 for an LED
PWMLed =18
GPIO.setmode (GPIO.BCM)
GPIO.setwarnings(False)
GPIO.setup(PWMLed, GPIO.OUT)
intensity = GPIO.PWM(PWMLed, 1000)
intensity.start(0)
intensity.ChangeDutyCycle(100)


interval = 1
frame = 0
# opens a preview screen for focusing the image and sets the shutter speed, turns off the auto gain and exposure

camera=PiCamera()
camera.resolution = (640,480)
camera.framerate=30
print("focus your camera")
camera.vflip=True
camera.start_preview(fullscreen=False, window =(1200,100,640,640))
camera.iso=100
```

```
time.sleep(2)
camera.shutter_speed=3555
camera.image_denoise=False
camera.shutter_speed=3555
print("shutter speed",camera.shutter_speed," (ms)")
camera.exposure_mode="off"
camera.awb_gains=(409/256,75/64)

g=409/256,75/64
print("awbgains",g)
camera.awb_mode="off"
camera.awb_gains=g
print(camera.awb_gains)

# This reports the gain settings for future reference
ag=camera.analog_gain
dg=camera.digital_gain
print("analog and digital gains", ag, ", ",dg)
```

*Python code for the calibration curve*

```
# User interface to determine the number of calibration images
print("enter the number of calibration point you want to record")
frames=int(input())
#print("enter the time you want to wait between frames in sec")
#print("needs to be longer than the shutter speed")
#mint=camera.shutter_speed/1000
#print(mint,"sec")
#tim=int(input())-mint

print("Choose the folder where you want to save your calibration images")
print("make sure your folder does not have spaces in the name")
path=askdirectory()
print(path)


#image capture loop
for i in range(frames):
    print("once you hit enter the device will record the image Automatically")
    print("Place your sample into the device, and enter the concentration for calibration image
#",i+1)
    imgconc=input()
    os.chdir(path)
    #imagetime=time.strftime("%m-%d-%Y_%H:%M:%S")
    camera.capture("CALB_"+imgconc+"_microM.jpg")
```

```
    #time.sleep(tim)


camera.stop_preview()

intensity.stop()
GPIO.cleanup()
sys.exit()

quit()
exit()
```

*Python code for Time-lapse images*

```
# User interface to determine the number of frames and timelapse interval
print("enter the number of frames you want to record")
frames=int(input())
print("enter the time you want to wait between frames in sec")
print("needs to be longer than the shutter speed")
mint=camera.shutter_speed/1000
print(mint,"sec")
tim=int(input())-mint

#User interface to set the file path for the recorded images
print("Choose the folder where you want to save your images")
print("Add your dye solution and then click OK")
path=askdirectory()
print(path)


#image capture loop
for i in range(frames):
    os.chdir(path)

    #imagetime=time.strftime("%m-%d-%Y_%H:%M:%S")
    camera.capture("image_frame_"+str(i+1)+".jpg")
    print("image number ",i+1," of ",frames," has been recorded")

    time.sleep(tim)


camera.stop_preview()

intensity.stop()
GPIO.cleanup()
sys.exit()
```

quit()
exit()

**Appendix B. Handout – student instructions**

You need to use two program packages to complete the data collection for your diffusion experiment.

Calibration curve data and Time-lapse collection of the diffusion process. Before you begin, you should make sure you have the following parameters determined.

1) The number of calibration points
2) The concentration of each calibration point
3) The number of images you want to record for your time-lapse capture of the diffusion experiment.
4) The length of time between each image capture of the diffusion experiment.
5) Where you want to save your files for each part of the data collection
    a) A couple of notes regarding saving files
        i) Raspbian tends to have issues with long file paths. Setting up a folder to save your files with a relatively short file path is recommended.
        ii) You can save your images in a folder in the Raspbian Suite or onto a USB flash drive.
        iii) Lastly, Raspbian also has issues with file paths and file names with spaces
            (1) Example of a file path that will most likely cause an error. "Timelapse Diffusion"
            (2) Instead, use "Timelapse-Diffusion"

If errors occur during the process, you can stop the script by hitting the stop button in the Thonny idle window. However, this only sometimes turns off the LEDs or camera.

If you will restart the script immediately, you can do so by hitting the run button.

If you do not restart soon, you should reboot the Raspberry Pi or turn it off by clicking the Raspberry Pi in the upper left corner and selecting logout.

*Initial setup of the device.*

Ensure the red wire is connected to the ground pin on the GPIO pins of the Raspberry Pi board and the green/black wire is connected to GPIO pin 18. For reference, see the Figure B.1 below.

These two provide power to the LED bank inside the box for illumination. The camera should already be connected to the Raspberry Pi Zero W.

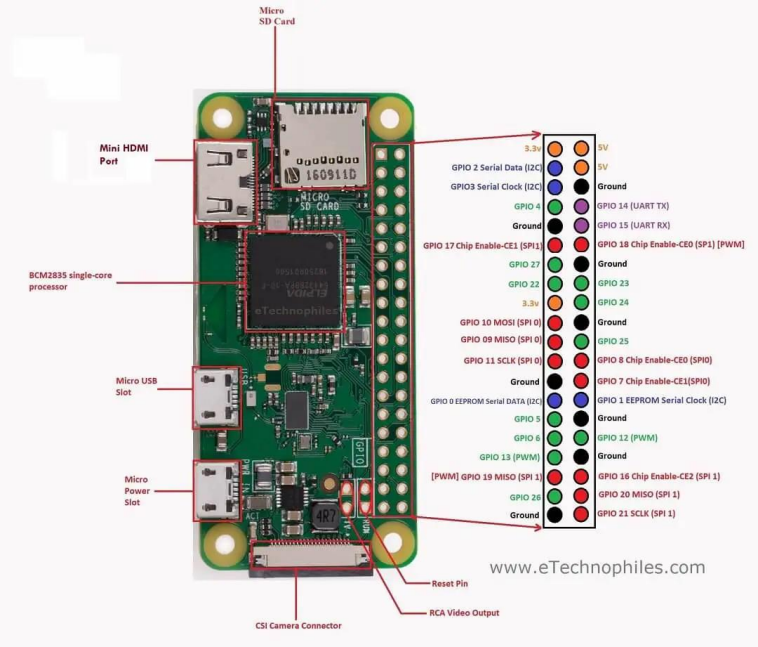



**Figure B.1.** GPIO diagram of the Raspberry Pi Zero W

*Calibration run*

1) double-click the test timelapse calibration file to start the calibration run.
2) A Thonny idle screen will open.
3) Once opened, click the run button.
4) The calibration software will start and provide some information and instructions in the shell window of the idle screen.
5) You should see the LEDs turn on. If not, check the connections on the GPIO board; the LEDs will not turn on if they are loose or not connected, as described above.
6) The program will open a camera preview screen. At this time, you can also focus the camera by turning the barrel inside the diffusion box. You should also make sure the image contains the most extensive view of the cuvette within the image. This can be adjusted by moving the camera bracket forward or backward as needed.
7) You will get a readout of the following specs in the idle screen shell window.
   a) Shutter speed
   b) Auto white balance gains (awbgains) as a fraction and decimal values
   c) And the analog and digital gains for your setup.
   d) The program should set these for your whole run.
8) It is recommended to record these values for both the calibration and diffusion runs, as any differences between the two programs may significantly impact your images. Moving the Thonny idle screen under the preview image is recommended while keeping the shell area visible. If not, when secondary dialog boxes appear, Thonny tends to place them under the preview image, making it difficult to access the dialog box.
9) You can start the calibration run once everything is adjusted.

a) Enter the number of calibration points you plan to record in the shell area of the Thonny idle screen and hit enter.
b) A dialog box will open asking where you want to save your image files.
c) You may use a folder on the home screen, connect a USB thumb drive, and select a folder.
d) Once a folder is selected, click OK.
e) Thonny will then ask you to place your desired sample into the diffusion imaging device and enter the dye concentration for that image.
f) Enter a numerical value of concentration in Micromolar only. Thonny will create the rest of the filename when it saves the image.
g) Once you are ready to take the image, hit enter.
h) Thonny will then ask you to place the following calibration sample into the device and enter its concentration. Once done, hit enter.
i) The process will repeat until you have completed the calibration samples you originally entered into the software.
j) Thonny will turn off the LEDs and the camera once it is completed.

*Time-lapse diffusion*

Once your calibration run is complete, you can set up your time-lapse diffusion experiment.

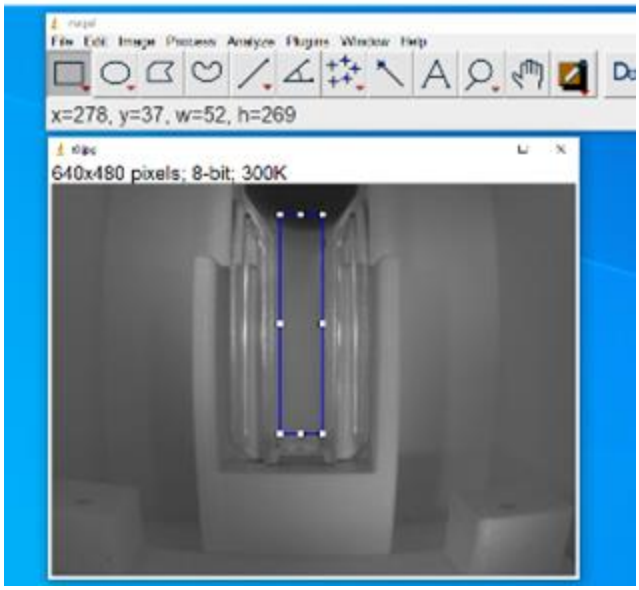
1) It is essential to ensure you don't move the camera or adjust the focus between the calibration run and the time-lapse diffusion.
2) Place your cuvette with agar gel into the diffusion device.
3) Double-click the "test timelapse V# with the self-setting parameters file. This will open a new page in the Thonny idle window.
4) Click run as before
5) Verify the parameters of the camera have not changed. Minor changes can occur but should be the same values as the calibration run less than 1 to 2% change.
6) Once everything is verified and running is in the calibration run (don't forget to move the idle window)
7) You can enter the number of images you wish to record for your time-lapse study and press enter.
8) Thonny will then ask for the time between frames of each image of the time-lapse in seconds. It must be a whole number and longer than the shutter speed.
9) Once input, hit enter.
10) select the folder where you want to save the data using the dialog box.
11) Thonny will create a filename for each image using a timestamp in the following format.
    a) Image_mm-dd-yyyy_hh.mm.jpg
    b) For example, if an image was recorded at 11:47 on 9/22/2023, the file name would be: Image_09-22-2023_11.47.jpg
12) The script will continue to run until all images are recorded. Once complete, Thonny will turn off the LEDs and camera.

**Appendix C. Image analysis**
The following steps are used:

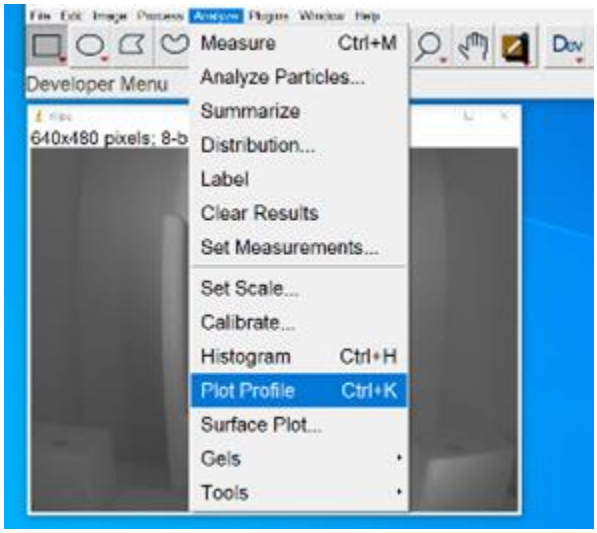
- Identify the region of interest (ROI) of images recorded during the time-lapse
- Convert the RGB image to an 8-bit greyscale.
- Compile the 2D pixel map into a 1D surface plot.
- Calibrating pixel size to the physical size of the object imaged.

1) Open the image file(s) slated for processing in ImageJ.
2) Convert the image to an 8-bit greyscale image by selecting Image>Type>8-bit.
    a) This function will compile the intensities of the red, green, and blue channels into one greyscale channel. The range of the colors in 8-bit varies from 0-255. Where 0 stands for black, 255 stands for white, and 127 stands for gray color.
3) Select the rectangle tool in ImageJ.

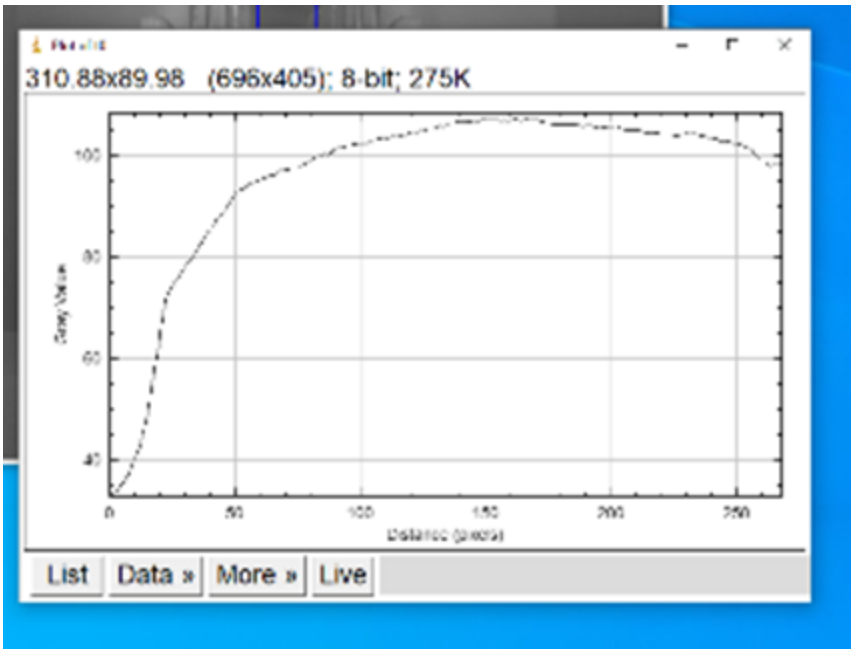

4) In the image, select the ROI for processing by clicking on the idea while holding down the left mouse button and dragging the ROI rectangle to encompass the area for processing.
    a) If several images are to be processed and the result is compiled into one data set, the RIO selection should be the same for every image.
        i) Before releasing the mouse during ROI selection, record the X and Y pixel location of the upper left-hand corner of the ROI and the Width and Height of the region selected.
        ii) If the mouse button is held down, this information will be displayed at the bottom of the ImageJ toolbar. See the example image below.




5) Select the plot profile function in Analyze> Profile Plot.



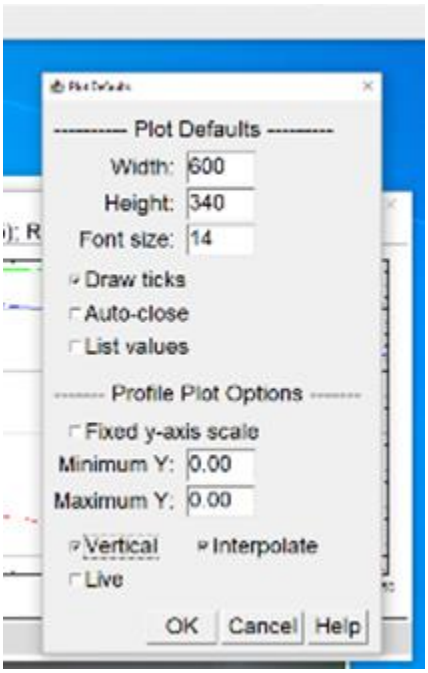

a) This function averages all pixel values in either the x or y direction and converts a 2-D image into a 1-D line. The default is to bin all pixels in the y direction. For this analysis, we will need to change the direction of binning to the vertical axis. This can be done by selecting more on the output graph.




b) At the bottom of the options, select Plot Defaults. Here, you can also choose to Set the range to fit all.


c) This will open the Plot defaults dialog box.

d) In the box, select the vertical option in the Profile Plot Options section and click OK.




e) If the vertical option is not selected, close the Plot window and rerun the surface plot.

f) If you are processing multiple images and don't close ImageJ, the Vertical option will not reset to the default.

6) To get numerical values, click the List option at the bottom of the plot. This will open a new window with the distance from the top of the ROI measured by the number of pixels and its average grey value.

7) You can copy and paste the two columns of data into any spreadsheet software you choose.

a) To create a calibration curve to average all the values in the ROI and use this value plotted against the concentration of the solution. Once all the calibration images are completed, Beer's Law should apply, and you should be able to find the equation of a best-fit line: Absorbance vs. Concentration.

i) Remember Abs = $\log(I_0/I)$, where $I_0$ should be the average value of the blank

b) For the timelapse images, see step 8.

8) To calculate a diffusion constant, the pixel size of the image needs to be calibrated to the actual physical dimension of the object. For a microcuvette, this can be done by physically measuring the window of your cuvette with a micrometer. (generally, microcuvette windows are ~4.5mm wide by ~23 mm tall). To determine the pixel equivalent, you can use the line tool in ImageJ to draw a horizontal line from one side of the window to the other, like the rectangle tool for selecting the ROI. Before releasing the left mouse button, ImageJ will show the number of pixels the line spans. This can be repeated vertically. The window is approximately 60 pixels wide and 300 pixels high for the images displayed here. But this may be different for your cuvette and pictures.

9) With both measurements, the physical distance a pixel represents is calculated in the following manner.
   a) distance/#of pixels = distance per pixel
   b) 4.5mm/60 = 0.075 mm or 75 micrometers
   c) 23mm/ 300 = 0.0767mm or 76.7micrometers

   To convert your grey scale values to the concentration, you should use the function created by your calibration curve in step 7a.