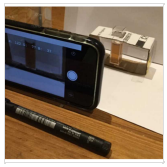

Jul 08, 2021

Mobile Phone Spectrophotometer Setup [Chlorophyll experiment version]

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Katharine Hubbard

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ABSTRACT

This protocol allows you to turn a mobile phone (smart phone) into a colorimeter/spectrophotometer. It goes through how to set up your phone and workspace, making up a dilution series, constructing a calibration curve and making measurements.

The protocol is designed so that it can be carried out independent of a laboratory - our undergraduate students used it as an at home experiment during the Covid-19 pandemic.

This version of the protocol is used to test the hypothesis "Chlorophyll content from sun leaves will be greater than shade leaves from the same plant".

The chlorophyll extraction requires you to have access to either 80% acetone (v/v with water) or an acetone based nail polish remover. Note that you will need to test whether your nail polish remover reacts with the plastic cuvettes or not before continuing with the experiment - some of our students found that their cuvettes dissolved using the nail polish remover directly.

The protocol is inspired by and adapted from this video - smartphone spectrophotometer

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PROTOCOL CITATION

Katharine E Hubbard 2021. Mobile Phone Spectrophotometer Setup [Chlorophyll experiment version]. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bwfspbne

KEYWORDS

mobile phone, smartphone, smart phone, spectrophotometer, colorimeter, at home experiments, chlorophyll

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MATERIALS TEXT

Mobile phone with camera
White card
1 cm path length cuvettes
50 mL centrifuge (falcon) tubes
3 mL plastic pipettes (pastettes)
Scissors
Sellotape
Marker pen
[optional] table lamp

Leaf material
Either 80% (v/v) acetone, or an acetone based nail polish remover

Cuvette holder template **cuvette holder design.pdf**

SAFETY WARNINGS

Acetone is flammable, so make sure there are no naked flames around your workspace

Setting up your phone

1 You first need to set up your phone to act as a colorimeter - video Instructions for this section are availabile: Setting up Your Mobile Phone as a Colorimeter

2 Choose a well lit space to work in. This protocol works better during the day under natural light. If you are doing the experiments after dark you might want to position a lamp near your set-up so you have fewer shadows and greater contrast in your images.

3 Fully charge your phone! Unplug your phone from it's charger and put the charger away to reduce the risk of electrocution while handling liquids.

4 Install a colour picker app on your phone - you need one that gives you numerical Red Green Blue (RGB) values. I recommend 'ColorPicker' for iPhones, and 'ColorGrab' for Android.

5 Download the template and make the cuvette holder - you will need sellotape to fix it together.
cuvette holder design.pdf

6 Sellotape the cuvette holder to the table/workshop where you are going to work. Place a piece of white card behind the cuvette holder. You might want to tape this into place too.

7 Open the colour picker app. Position your phone so that you are pointing the colour picker pointer in the middle of your cuvette. I would recommend resting your phone against something (e.g. a mug/box) so that it stays in the same position for each of your measurements.

My set-up is shown below - note for these pictures the cuvette is filled with a tea solution not chlorophyll!

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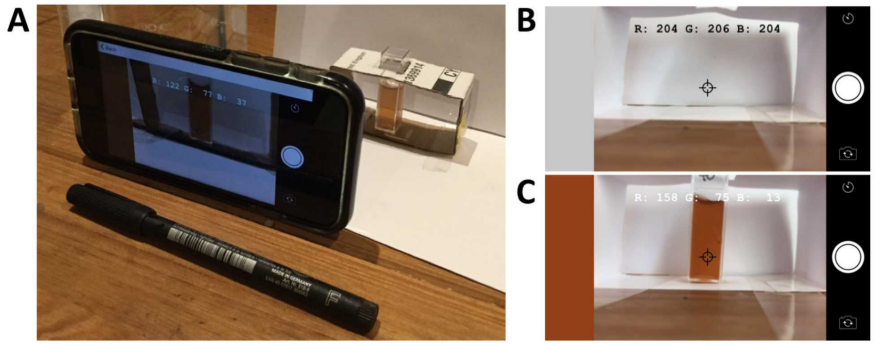

A: Relative positions of the phone, cuvette holder and cuvette. B: Screenshot of the setup without a cuvette (R:204,G:206,B:204). C: Screenshot of the setup with a cuvette of 50% diluted tea (R:158,G:75,B:13).

Calibration curve (chlorophyll experiment)

8 To test that your phone is able to measure different concentrations you will need to set up a calibration curve. For this you will construct a dilution series of different concentrations, and measure the RGB values for each concentration. You then plot this on a graph to determine if there is a relationship between concentration and RGB.

The calibration curve is to test whether or not the phone is able to measure a relationship between concentration and RGB values - it is a test of your phone, not of your experimental samples.

Video instructions for chlorophyll extractions and calibration curve

9 You will extract chlorophyll using nail polish remover. You need to use a brand of nail polish remover that is acetone based - read the ingredients before purchasing some! If you have access to a lab you can use 80% acetone for the extractions.

⚠ Acetone is flammable, so make sure there are no naked flames anywhere around your workspace

10 Before starting, you need to test whether your brand of nail polish remover reacts with the plastic cuvettes - brands with very high acetone content will start to dissolve the cuvettes.

Put 3 mL of nail polish remover into a cuvette and leave it for 10 minutes.

- If the plastic stays clear you can use the nail polish remover directly.
- If the plastic goes cloudy or deforms you will need to dilute the nail polish remover for your experiments. We suggest an 80% dilution e.g. 40 mL nail polish remover mixed with 10 mL water.

11 Pour 20 mL of nail polish remover into a plastic falcon tube labelled 'Chlorophyll stock'

12 Get ~8 medium sized leaves (I used spinach leaves) and cut them up into small pieces (I used 0.5 cm squares). Push them into the falcon tube so they are immersed in the acetone and put the lid on.

13 Leave the Chlorophyll stock for ~30 minutes to let the chlorophyll dissolve into the acetone

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14 Pipette 2.5 mL of the extract into a new 50mL tube. Add 22.5 mL of acetone so you have a total of 25 mL. Label this as '10% chl stock'

15 Using a plastic disposable pipette, set up a dilution series of different concentrations of chlorophyll in the cuvettes. I suggest you make up cuvettes with the following concentrations. Make sure you label your cuvettes clearly at the top or on the frosted side.

Note that you are making up your dilutions with nail polish remover, not water. Most nail polish removers have a coloured dye in them so you need to take this into account when preparing your dilution series.

A	B	C
Concentration of chlorophyll	Volume of chlorophyll stock solution	Volume of nail polish remover
100%	3 mL	0 mL
80%	2 mL	0.5 mL
60%	1.5 mL	1 mL
40%	1 mL	1.5 mL
20%	0.5 mL	2 mL
	Volume of 10% chlorophyll stock	Volume of nail polish remover
10%	3 mL	0 mL
5%	1.5 mL	1.5 mL
0%	0 mL	3 mL

Volumes required to make up dilutions series

16 Place the 100% cuvette into the cuvette holder with the transparent side facing towards you. [Note that the cuvettes have a clear side and a frosted side]

17 Load up the color picker on your phone and record the RGB Values for the 100% cuvette.

18 Repeat for each of your dilutions.

19 Plot a calibration curve with concentration on the x-axis and measured RGB value on the y-axis. Mine is shown below - you will notice that there is a negative relationship between RGB and concentration. This is because on the RGB scale white = 255 and black = 0.

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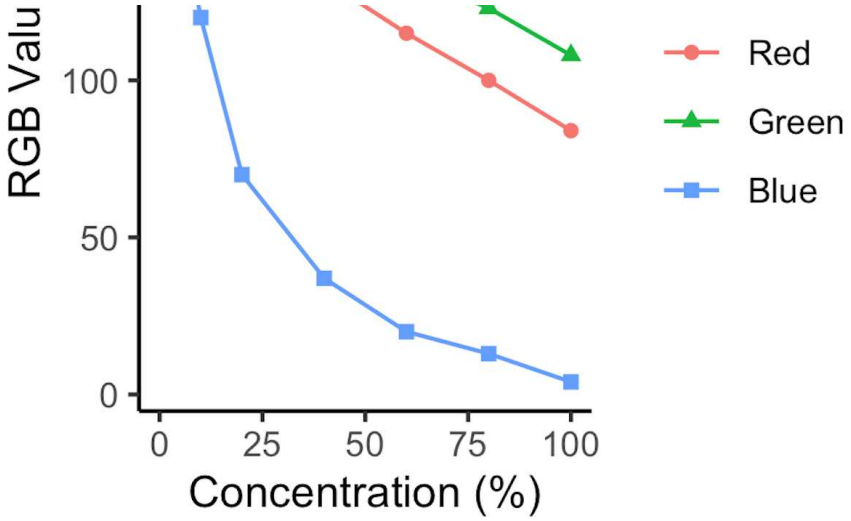


My chlorophyll calibration curve, using raw RGB values from the colorpicker app

20 To give a positive relationship between concentration and RGB I recommend that you normalise your data as follows:

$$normalised.value = 1 - (RGBvalue/255)$$

For example, if you measured R as 86, you would calculate your normalised value as:

$$normalised.value = 1 - (86/255) = 1 - 0.337 = 0.663$$

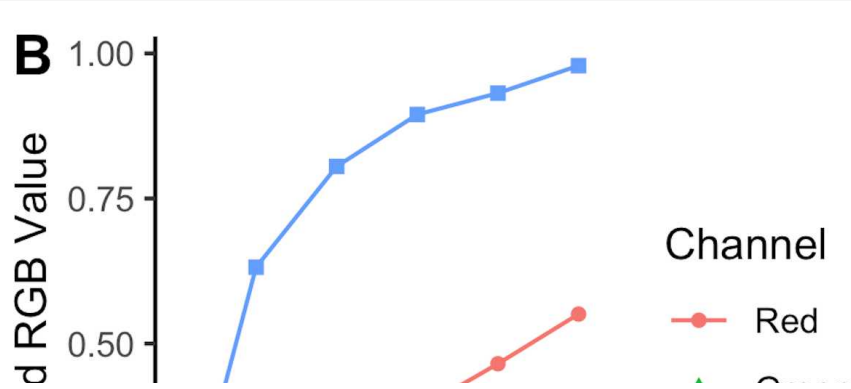
Alternatively, you could calculate this relative to a blank (empty) cuvette, which would be a better approximation for a spectrophotometer. This would be calculated as follows:

$$normalised.value = 1 - (sample.RGBvalue/blank.RGBvalue)$$

For example, if you measured B as 120 for your sample, and 190 for your blank you would calculate the normalised value as:

$$normalised.value = 1 - (120/190) = 1 - 0.631 = 0.368$$

21 Plot a second calibration curve with the normalised values. Mine is below using the blank normalisation calculation. - You will see that this normalisation has given a positive relationship between concentration and normalised RGB. Normalised RGB is now scaled so that 0 = white and 1 = black.



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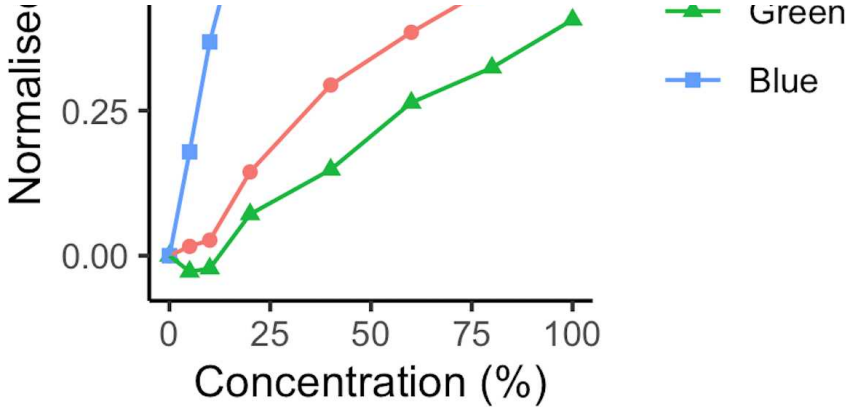

My normalised calibration curve. Note that I used the blank normalisation calculation, hence I got some negative values for the green channel

Methodology Decisions

22 **Decide which colour channel you are going to use for the rest of your experiments.** Pick the channel which gives the best relationship between concentration and RGB. For example, in my chlorophyll experiment I would pick the Blue channel as this has the strongest relationship between concentration and RGB value.

Experimental Measurements

23 Put 3 mL of nail polish remover into a fresh cuvette.

24 Cut out 3 cm^2 of leaf material from your chosen plant. Cut this into small pieces and place into the cuvette - push the pieces so they are fully submerged in the nail polish remover.

25 Leave for 20 minutes to let the chlorophyll diffuse into the acetone.

26 Transfer the acetone into a fresh cuvette, leaving the leaf material behind. You could do this by pouring the liquid into a new cuvette, or by pipetting it across.

27 Put the cuvette into the cuvette holder and measure the RGB for your chosen colour channel using the colour picker app, and record your data.

28 Repeat for all replicates in your experiment. Make sure you use the same incubation time for each replicate, and try and cut the leaf material into similar sized pieces so that you are consistent between samples.

If you want to perform a t-test, you will need at least 5 replicates of each sample (i.e. 5 extractions from sun leaves, 5 extractions from shade leaves).

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