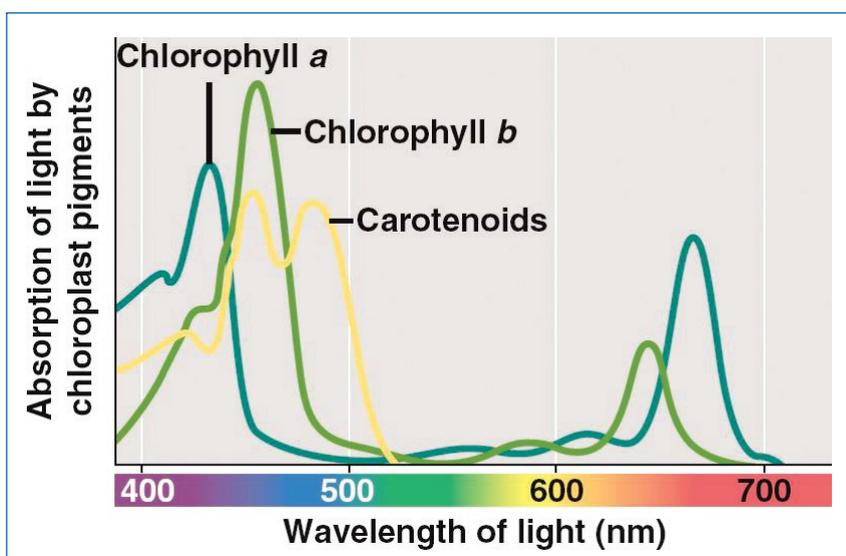


# Digitising an image

A (possibly sad...) member of the SSERC Biology Team recently wanted to obtain the absorption spectrum of chlorophyll b in digital form. There are a number of ways by which this data set could be generated.



**Figure 1** - The absorption spectra of chlorophyll a, chlorophyll b and 'carotenoids' (adapted from <http://www.glogster.com/austinprice2012/light-absorption-in-chloroplast/g-6ksfuqsdk18i206c2tmc1a0> - accessed August 28<sup>th</sup> 2013).

Colorimeters, generally, are not suitable for the task since they do not offer sufficient observation wavelengths to allow good resolution. Additionally the standard cuvettes used in colorimeters and spectrophotometers are not very resilient to the 'normal' solvents (hexane, propan-2-one etc.) for chlorophyll.

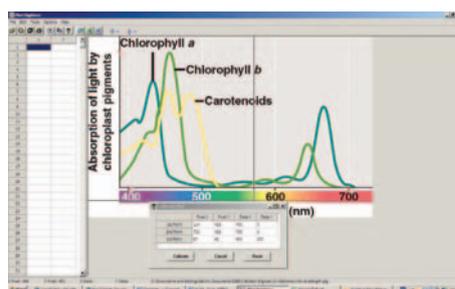
The search, as is often the case, then moved to the web as a possible source of data. Entering the phrase 'chlorophyll absorption spectrum' into Google Images produces a plethora of wonderful images which can be downloaded. A typical image is shown in Figure 1.

The challenge now is how do you generate a set of data points which characterise each of the various curves shown in Figure 1?

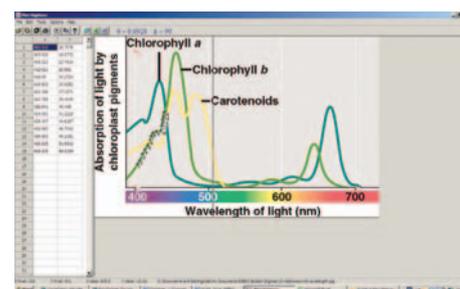
Alternatively is it possible to generate a set of (x,y) coordinates for the curves? One option open to us is to use a graph paper/ruler combination. It is, of course, possible to download the image, increase its physical size and print it out and then with judicious use of a ruler (possibly a set-square?) generate a set of data points. Suppose you want data

approximately every 2 - 4 nm this becomes a laborious process but clearly it can be done. An alternative approach suggested (somewhat wistfully it has to be said) by one of the more 'experienced' members of the team was to see if we still had an epidiascope that would project the image on to a wall or screen onto which a grid had been painted. The fact that many of the available walls in SSERC have recently been painted made this option a non-starter although we might still have an epidiascope.

The question which we then asked was 'Is there a piece of readily available, simple, cheap (free would be even better!) and reliable software that would do the job for us?' The obvious place to look for an answer is the web and the search term 'digitize plot' (with some reluctance we avoided the spelling 'digitise') yielded a number of possibilities. For a variety of reasons we were attracted to a piece of software called 'Plot Digitizer' which is available as a free download (<http://www.southalabama.edu/physics/software/plotdigitizer.htm>) from the Physics Department at the University



**Figure 2** - Calibration screen for the jpeg image from Figure 1. Software used was 'Plot Digitizer' (available at: <http://www.southalabama.edu/physics/software/plotdigitizer.htm>).



**Figure 3** - Partial digitisation of the jpeg image from Figure 1. Software used was 'Plot Digitizer' (available at: <http://www.southalabama.edu/physics/software/plotdigitizer.htm>).

of South Alabama. The software is described as 'a useful program for extracting data from a linear, semi-log, or log-log plot'. Apparently all you need to do is 'Using an optical scanner, create a Bitmap or JPEG image of the plot and open the image file in Plot Digitizer. Then, after calibration, you can extract the data values by merely clicking on the data points'.

This sounded as if it would fit the bill. At first glance it is readily available, cheap and simple. The remaining question centres on reliability. The steps involved in digitisation are:

- Import an image file (we used a jpeg version of figure 1).
- Calibrate the screen - i.e. you need to 'tell' the software the (x,y) coordinates of 3 points on the plot

which you wish to digitise (see Figure 2). The points chosen were (400, 0), (700, 0) and (400, 100) - the latter being an arbitrary value given that the plot in figure 1 does not display values on the y-axis.

- Using a mouse (or keypad), trace the curve and at relatively frequent intervals click the mouse and the coordinates of the point will be recorded in the data columns (Figure 3).
- Once all data points have been collected the file can be exported in a variety of formats the most useful of which we found to be as a Microsoft Excel file.

Having digitised the curve for the chlorophyll b absorption spectrum using the software and generated an Excel file of the data, we are now in

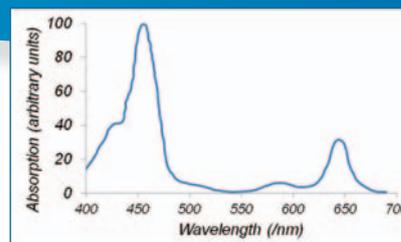


Figure 4 - Absorption spectrum for chlorophyll b generated by digitisation of the curve in Figure 1. The curve shows data from 120 points generated using 'Plot Digitizer' (available at: <http://www.southalabama.edu/physics/software/plotdigitizer.htm>).

a position to address the remaining question 'How reliable is the software?' Figure 4 shows a plot of absorption vs wavelength for the chlorophyll b curve from Figure 1.

In an ideal world the plot in Figure 4 would be identical to the chlorophyll b curve in Figure 1 and it looks pretty good to us. Our conclusion is that Plot Digitizer 'does what it says on the tin'.

## Health & Safety

# Breaking bad bottles

We have had a few reports recently concerning the structural failure of plastic bottles. While this can often be a mere annoyance, the bottles in question have been ones containing concentrated acids.

In one case a bottle of concentrated sulfuric acid was picked up by the moulded handle which promptly broke off. In another, a bottle of nitric acid was picked up by the neck and it broke there. There have been similar reports from our colleagues in England as well.

As far as we can ascertain, these are the original bottles the chemicals came in from the suppliers. They are also usually a few years old, 4 to 6 or so. We should add here that the reports concern bottles from a variety of suppliers.

Having looked into the subject, it seems that plastics of various sorts are prone to embrittlement, albeit at a slow rate, usually as a result of the plasticiser leaching out into whichever solution is being kept in them. This process can be accelerated by various factors, depending on the particular plastic involved. These include heat, uv light and oxidising agents.



Figure 1 - A nitric acid bottle showing severe cracking.

This is still an uncommon problem but we would recommend that if you are handling a plastic bottle of concentrated acid you inspect it beforehand and handle it carefully. In the longer term, if you find you have bottles of, for instance, concentrated nitric acid, that tend to sit around for several years, it might be an idea to see if it is possible to purchase in smaller quantities (1 litre rather than 2.5 litres for example) to make sure that the bottles are not sitting in your chemical store for too many years.