# Chlorophyll

## Extraction

### Materials

- ☐ Acetone (2 mL per sample)
- ☐ Centrifuge with rotor 12154
- ☐ Ice
- ☐ 1,000 µL pipette and tips (2 tips per sample)
- ☐ Vortexter
- ☐ Centrifuge
- ☐ Kimwipes

### Method

> **Caution**
>
> Chlorophyll is very unstable. Always keep samples on ice and as dark as possible.

1. Centrifuge up to 24 sample tubes using rotor 12154 for 5 min at 12,000 rpm at 4℃. Keep centrifuge balanced if less than 24 tubes are used.
2. Remove supernatant with 1,000 µL pipette. Carefully remove remaining drops of supernatant with Kimwipes.
3. Add 1,000 µL acetone and close tube. Homogenize with pipette and vortex for 15 s. Do not re-use tips.
4. Extract chlorophyll for 24 h at 4 ℃ (Fridge Biomol 2). Keep samples wrapped in aluminium foil.

## Measurement

### Overview

The chlorophyll concentration ($g\ mL^{-1}$) is calculated based on the formulas given in (Jeffrey & Humphrey, 1975):

$$\text{Chlorophyll } a = 11.43\frac{A_{663} - A_{750}}{PL} - 0.64\frac{A_{630} - A_{750}}{PL}$$

, where $A_{663}$, $A_{630}$, and $A_{750}$ is the absorption at 663, 630, and 750 nm, and $PL$ the path length of the cuvette (1 cm).

The photometer can read whole plates (96 samples). However, since acetone is used to extract the chlorophyll, a quartz cuvette has to be used instead of plates made of plastic. The software of the photometer still visualizes the measurements of this cuvette as a plate which can be confusing.

The photometer will automatically measure the absorption at 663, 630, and 750 nm and does the blank-correction automatically. Each measurement is done twice and blanks are measured at the beginning and after every 10 samples, resulting in the following plate outlay:

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | BLK | BLK | SPL1 | SPL1 | SPL2 | SPL2 | SPL3 | SPL3 | SPL4 | SPL4 | SPL5 | SPL5 |
| B | SPL6 | SPL6 | SPL7 | SPL7 | SPL8 | SPL8 | SPL9 | SPL9 | SPL10 | SPL10 | BLK | BLK |
| C | SPL11 | SPL11 | SPL12 | SPL12 | SPL13 | SPL13 | SPL14 | SPL14 | SPL15 | SPL15 | SPL16 | SPL16 |
| D | SPL17 | SPL17 | SPL18 | SPL18 | SPL19 | SPL19 | SPL20 | SPL20 | BLK | BLK | SPL21 | SPL21 |
| E | SPL22 | SPL22 | SPL23 | SPL23 | SPL24 | SPL24 | SPL25 | SPL25 | SPL26 | SPL26 | SPL27 | SPL27 |
| F | SPL28 | SPL28 | SPL29 | SPL29 | SPL30 | SPL30 | BLK | BLK | SPL31 | SPL31 | SPL32 | SPL32 |
| G | SPL33 | SPL33 | SPL34 | SPL34 | SPL35 | SPL35 | SPL36 | SPL36 | SPL37 | SPL37 | SPL38 | SPL38 |
| H | SPL39 | SPL39 | SPL40 | SPL40 | BLK | BLK | SPL41 | SPL41 | SPL42 | SPL42 | SPL43 | SPL43 |

BLK corresponds to blank measurements and SPLXX to samples.

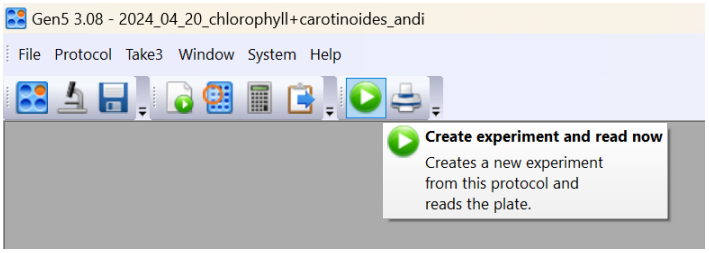
> **Note**
>
> The photometer automatically blank-corrects the measurements which can lead to wrong measurements if the order of samples and blanks differs to the expected order. It is therefore important to note the actual order of the samples in the metadata sheet (described here).

Materials

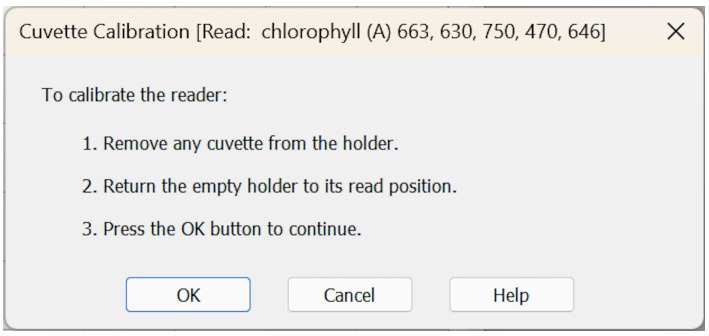
- ☐ Vortexter
- ☐ Centrifuge with rotor 12154
- ☐ Ice
- ☐ Acetone
- ☐ Kimwipes
- ☐ 1000 µL pipette & tips
- ☐ BioTek Epoch2 microplate reader
- ☐ Quartz cuvette (dark and shattered)

Method

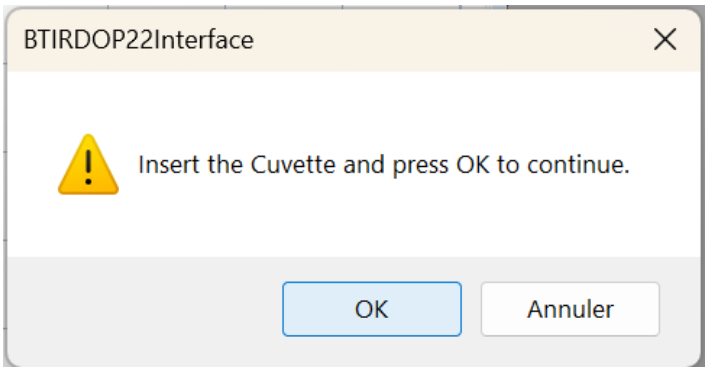
1. After the incubation (note down incubation time), vortex samples for 15 s and centrifuge samples using rotor 12154 for 5 min at 12,000 rpm at 4 °C. Keep samples on ice and in darkness. Transport samples carefully to not damage the pellet.
2. Turn on BioTek photometer and connect to Dell via USB.
3. Double-click on `2024_04_20_chlorophyll+carotinoides_andi.prt` to start software (backup stored here).
4. Click on `Create experiment and read now`:



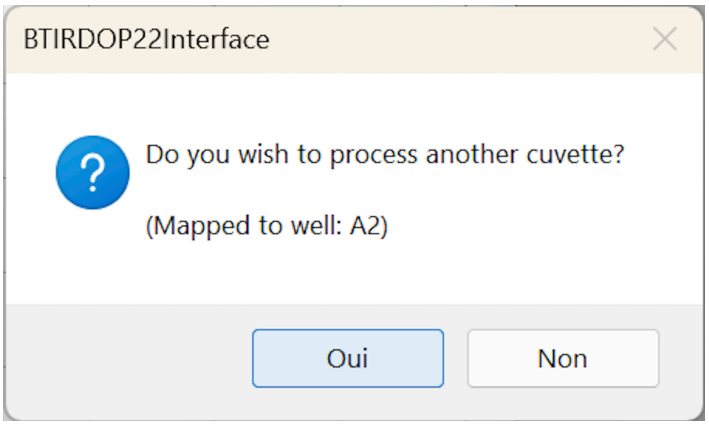
5. At first, the photometer takes a background measurement without any cuvette inserted. Follow the instructions in the pop-up and click `OK`.




6. Start measuring the blank. Clean quartz cuvette with acetone and Kimwipes, add 500 µL acetone, and insert cuvette into photometer. Click `OK` in pop-up window.




7. Repeat blank measurement. Click on `Oui` in pop-up window.

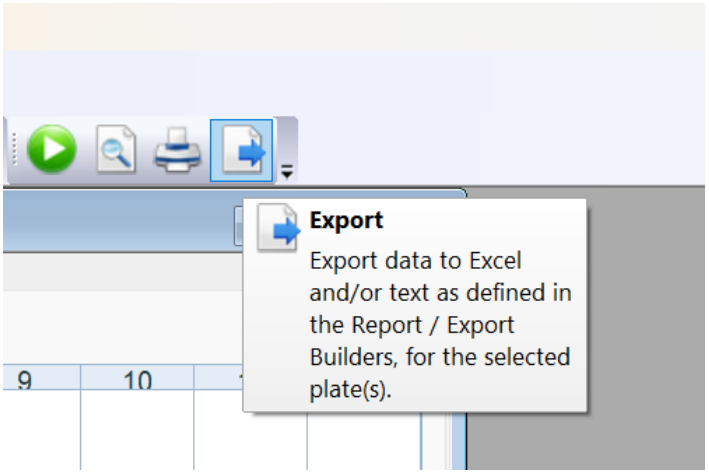



8. Measure first sample. Remove cuvette from photometer, clean with acetone and Kimwipes and add 500 µL sample extract. Click on `Oui` in pop-up window (as for second blank measurement).
9. Repeat measurement (click `Oui`).
10. Remove cuvette from photometer, clean with acetone and Kimwipes and measure next sample.
11. Repeat as described before for 10 samples (i.e. 20 measurements). Then, measure blank as described before (2x) and continue to measure samples. Take care not to damage the pellet.

> **Note**
>
> If you do a mistake in the sequence of measurements, correct it accordingly in the metadata sheet.

8. When all samples are measured or after 43 samples, click on `Non` in pop-up window.
9. Save experiment in your folder. Name it as follows: `YYYY_MM_DD_chl_adaptome_#.xpt`, where `YYYY_MM_DD` is the date and `#` the batch number (each day, start with 1), e.g. `2024_11_19_chl_adaptome_1.xpt`.

10. To export the data, click on `Export`.




11. Save data as `.txt` (default) in your folder and name it `YYYY_MM_DD_chl_adaptome_#.txt` (as described above).

> **Caution**
>
> Backup your data each day you are measuring. Either upload the folder to Google Drive or save it on a USB key. Keep all data also on the computer.

Jeffrey, S. W., & Humphrey, G. F. (1975). New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. *Biochemie Und Physiologie Der Pflanzen*, *167*(2), 191–194. https://doi.org/10.1016/S0015-3796(17)30778-3