

# Calculate chlorophyll a concentration and Symbiodiniaceae counts

If the metadata sheet is filled out correctly, R functions, stored in a package called `coralchlo`, can be used to calculate the chlorophyll a concentration with the absorption measured with the photometer. The coral fragment area is calculated with the change in weight before and after adding the second layer of paraffin wax and used to normalise the chlorophyll a concentration and Symbiodiniaceae counts per area.

The package can be installed with the `devtools` package:

```
devtools::install_github("andieich/coralchlo")
```

The current version is 0.0.0.2.

After installation, you can load the package and download the metadata sheet. During this the import of the sheet, some basic test are done to ensure it was filled out correctly and that the values make sense.

```
library(coralchlo)

link_metadata_sheet <- "example_link"

dat_overview <- read_metadata(link_metadata_sheet)
```

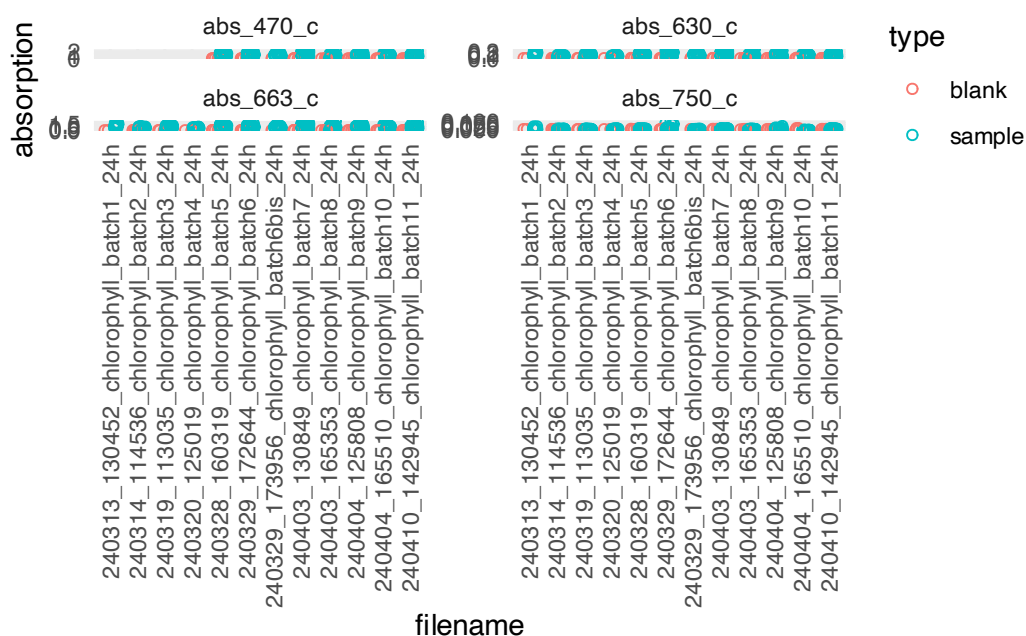
Then, the chlorophyll a concentration is calculated based on the absorption measured with the photometer.

The photometer files (.csv) can be store in Google Drive or locally. If they are stored locally, `path_to_biotekfolder`, is the path to the folder containing these files. If they are stored in Google Drive, `path_to_biotekfolder` is the path within the Google Drive to the folder containing the photometer files. If you use Google Drive, set `is_googledrive = TRUE`. The files are downloaded to a temporary folder or to a folder specified in `download_directory`. All files in `download_directory` will be replaced.

The values are blank-corrected. To ensure that the blank values make sense, they are plotted for all wavelengths and photometer files. This can be skipped by setting `plot = FALSE` in the `normalise_chl_per_area()` function. The area data is used to normalize the chlorophyll a concentration for the surface area of the coral fragment. For each sample, two measurements were taken, therefore two chlorophyll a and c2 concentrations as well as their sum will be exported.

```
dat_chl <- normalise_chl_per_area(dat_overview,
                                path_to_biotekfolder = "example_path")
```

sample_id	measurement_replicate	chl_a_per_cm2	chl_c2_per_cm2	chl_tot_per_cm2
2TSML27P	m1	3.416571	0.4155315	3.832102
2TSML27P	m2	3.416571	0.4155315	3.832102
2TSPL07P	m1	2.741200	0.3220933	3.063293
2TSPL07P	m2	2.747538	0.3200805	3.067618
2TSAL09P	m1	5.251357	0.5499048	5.801261
2TSAL09P	m2	5.251357	0.5499048	5.801261
2TSML26P	m1	6.467369	0.5805320	7.047901
2TSML26P	m2	6.467369	0.5805320	7.047901
2TSAL07P	m1	6.488811	0.6076038	7.096414
2TSAL07P	m2	6.488367	0.6264047	7.114771



```
head(dat_chl, n = 10)
```

Similarly, the Symbiodiniaceae counts are normalized for the surface area of the coral fragment. Since six measurements are taken per sample, six values are exported

```
dat_counts <- normalise_counts_per_area(dat_overview)
head(dat_counts, n = 10)
```

sample_id	count_replicate	count_per_cm2
2TSML27P	c1	4924526
2TSML27P	c2	4559746
2TSML27P	c3	5471696
2TSML27P	c4	4377357
2TSML27P	c5	5654086
2TSML27P	c6	4742136
2TSPLo7P	c1	5267582
2TSPLo7P	c2	6099306
2TSPLo7P	c3	5128962
2TSPLo7P	c4	4297238