

# 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 0.0.0.1.

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_metadatasheet <- "example_link"

dat_overview <- read_metadata(link_metadatasheet)
```

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

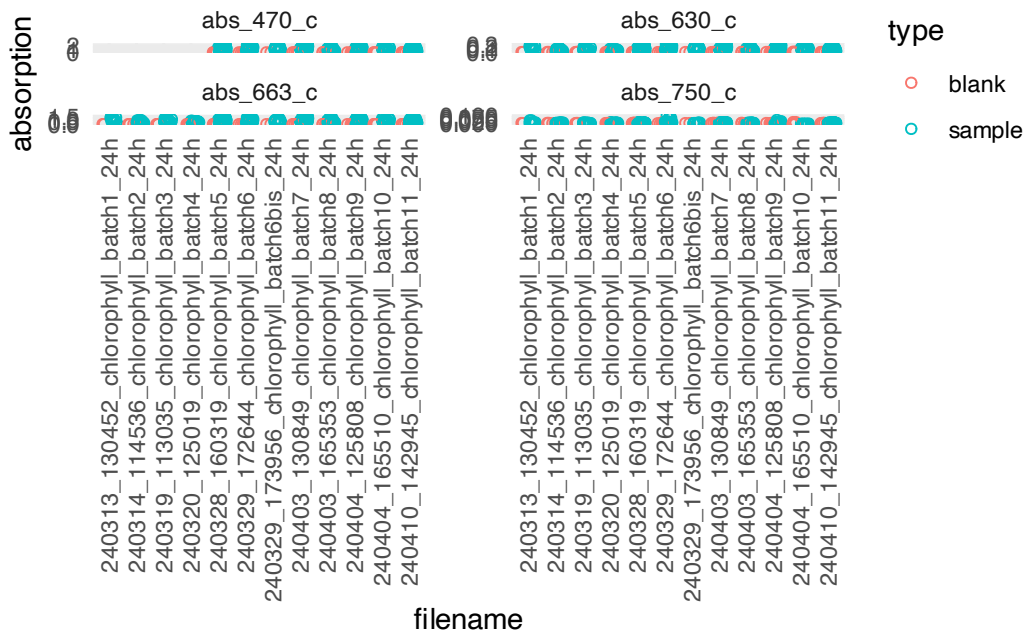
The photometer files (`.csv`) can be store in Google Drive or locally. If they are stored locally, `path_to_biotekfolder`, is the path to the folder containing these files. If they are stored in Google Drive, `path_to_biotekfolder` is the path within the Google Drive to the folder containing the photometer 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_chla_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 concentrations will be exported.

```
dat_chla <- normalise_chla_per_area(dat_overview,
                                   path_to_biotekfolder = "example_path")
```

sample_id	measurement_replicate	chl_a_per_cm2
2TSML27P	m1	3.416571
2TSML27P	m2	3.416571
2TSPLo7P	m1	2.741200
2TSPLo7P	m2	2.747538
2TSALo9P	m1	5.251357
2TSALo9P	m2	5.251357
2TSML26P	m1	6.467369
2TSML26P	m2	6.467369
2TSALo7P	m1	6.488811
2TSALo7P	m2	6.488367

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



```
head(dat_chla, 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)
```