

Examination of Chlorophyll *a* Autofluorescence Level within Marine Diatoms

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ABSTRACT

Red chlorophyll auto-fluorescence refers to the emission of fluorescent light when high energy UV light was shined to chlorophyll *a* molecule. This specific type of auto-fluorescence was present in diatoms, but the intensity of auto-fluorescence varies with species. This study evaluates the relative auto-fluorescence intensity for diatoms taken from Dapeng Bay, Shenzhen. The auto-fluorescence intensity in *Biddulphia mobiliensis* was relatively high probably due to lower photosynthetic efficiency with high level of chlorophyll *a*; Other species, such as *Melosira* sp. and *Skellonema* sp. examined in this study demonstrated lower auto-fluorescence intensity as they probably have more efficient photosynthetic processes and possess less overall number of chloroplasts.

Introduction

Auto-fluorescence is the natural emission of light by biological structures when they have absorbed light (Monici, 2005). It is a widespread phenomenon that particularly occurs in plants and algae during photosynthesis. The mechanism of fluorescent light is induced in photosynthesis when chlorophyll absorbs a specific range of light. The high energy photons in the light let the electrons in chlorophyll molecules gain energy and boost them to an excited state with higher energy level. However, unstable high energy excited state was unable to be maintained, thus the high energy electrons in the excited state will return to their original orbital, or the ground state, by releasing the excessive energy as heat and photons in specific wavelengths (Fig. 1). This afterglow of photons was called fluorescence. Specifically in plant species, the fluorescence appears red, a wavelength between 620-700nm.

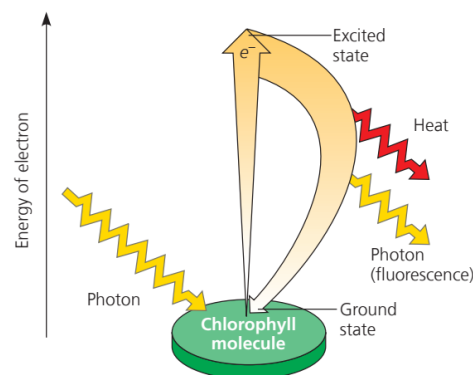


Figure 1. Fluorescence mechanism in chlorophyll molecules (Urry et al. 2019)

Measurement of fluorescent light has been proven to be an effective tool in measuring chlorophyll concentration and the efficiency of PSII (Photosystem II). Previous study has demonstrated a linear relationship with fluorescence amount with chlorophyll *a* concentration (Matorin et al. 2004); The concept of fluorescence induction and

Kautsky effect could be utilized to analyze photosynthetic and particularly PS II efficiency. In this study, we analyzed the auto-fluorescent level of estuary and oceanic coastal diatom species obtained from phytoplankton sampling of the estuary Shenzhen Bay and the oceanic Mirs Bay.

Material and Methods

Sampling Sites

The sampling sites of marine and freshwater Diatoms take place at the Daya Bay at southern coast of China (22.564922, 114.535567) and Dasha River discharge point into Shenzhen Bay (22.519748, 113.955440). Marine Diatoms were sampled at the Daya Bay on 21/12/2022 afternoon at 2p.m. to 4p.m..The pH of the sample site was 8.4 and the salinity level was 32 ppt. Both fresh and marine diatoms were sampled at the discharging point at Dasha River Dam on 26/12/2022 afternoon at 1p.m. to 2p.m. Plankton samples were collected at freshwater point where the salinity was measured as 2.5 ppt and at the river water discharge point where salinity was measured as 22.5 ppt.

Sample collection and treatment

Both marine and freshwater phytoplankton were collected with plankton net of 300 mm frame and 35 μ m mesh. Concentrated phytoplankton samples were collected by tossing plankton net about 1 meter away into the water body and pulling it back at slow speed for five times. The total volume of sampled phytoplankton was stored in three separate brown plastic sampling bottles, labeled by location and salinity. Plastic bottles were transferred to a 4°C fridge to keep the algae fresh and slower the metabolism.

Infrared Microscopy

Approximately 10 μ l of samples of diatoms were extracted by pipet onto a glass slide. Image acquisition was carried out with the SopTop fluorescent microscope (Sunny Optical Technology Co.,LTD) and a CCD 8.3MP 1/1.2" CMOS Senso). The 40 \times objective was used. The light source for fluorescence excitation was generated by an Sony Exmor Camera. Fluorescence filters were applied to observe auto-fluorescence in diatom. The excitation filter was an EX350/50nm and the emission filter was a 575nmLP, with a dichroic reflector 400nm.

Specific diatoms were first identified by white light and photographed using the microscope set at 40x. Once the diatom was properly focused, the white light was switched off, and the shutter was closed. Then the fluorescent filter, allowing UV light (350nm) to pass through and induce chlorophyll *a* auto-fluorescence in diatoms. The red light emitted by chlorophyll *a* auto-fluorescence was observed by gradually increase exposure time and gain till 450ms and 1200%. A photograph was taken with these parameters. The two photos taken per diatom species were saved and named properly by species and sampling cite. Each distinct diatom sample selected was treated the same, and only one sample was analyzed at one time. A total of 73 samples of both fresh and marine diatoms were analyzed.

Data Treatment

The pictures were further analyzed with Image J. Using split channel analysis, the area of the auto-fluorescence area can be calculated with maximum, minimum, and mean values. Species were further identified with specific specie names or general specie names (Yang and Dong, 2006; Deng, 2012; Krammer and Lange-Bertalot, 2011), and their average value of chlorophyll *a* auto-fluorescence were calculated.

Results

A total of 16 diatom species were recorded. The species included *Chaetoceros* sp., *Coscinodiscus* sp., *Ditylum brightwellii*, *Lephtocylindrus danicus*, *Licmophora* sp., *Melesira* sp., *Navicula* sp., *Nitzschia* sp., *Odontella* sp., *Pinularia* sp., *Pseudo-nitzschia delicatissima*, *Rhizosolenia* sp., *Skeletonema costatum*, *Skeletonema* sp., *Synedra* sp., and *Thalassionema nitzschioides*. The data were generated by 64 of the 73 marine phytoplankton pictures that were identified as specific diatoms.

In general, all species showed red auto-fluorescence under the excitation of blue wavelength (Fig. 2). Analysis of the average mean value of chlorophyll a auto-fluorescence intensity in various marine diatom species in both samples combined showed the strongest chlorophyll a auto-fluorescence intensity in an observed *Navicula* species while the weakest in *Melesira* species (Fig. 3). The result could imply potential positive relationship between chlorophyll a concentration to chlorophyll a auto-fluorescence intensity in the examined *Navicula* species.

Intraspecies variation in chl. a red fluorescence intensity variation was recorded (Fig. 4). *Odontella* species demonstrated the greatest variation among each sample recorded, and *Skeletonema costatum* demonstrated the least.

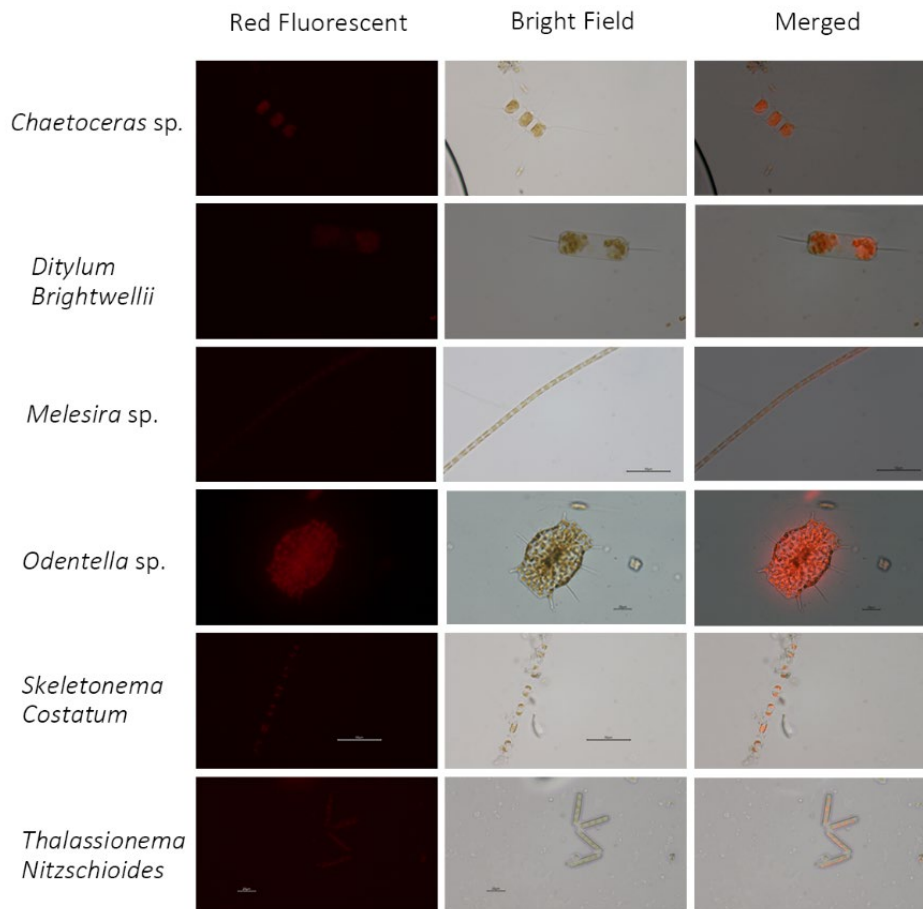


Figure 2. Representative pictures of Diatoms and their chl. a auto-fluorescence signal pattern.

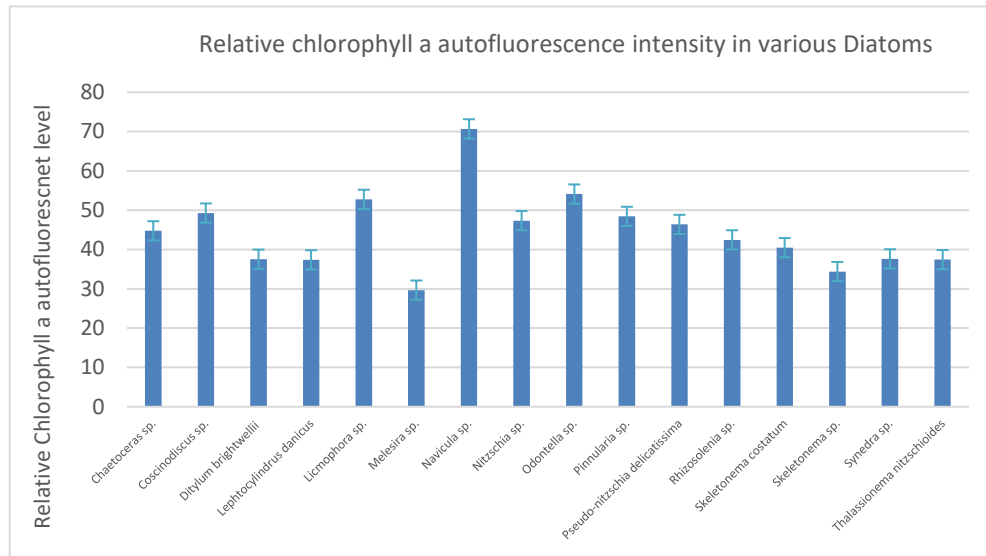


Figure 3. Relative chlorophyll *a* auto-fluorescence intensity in all the observed diatom species.

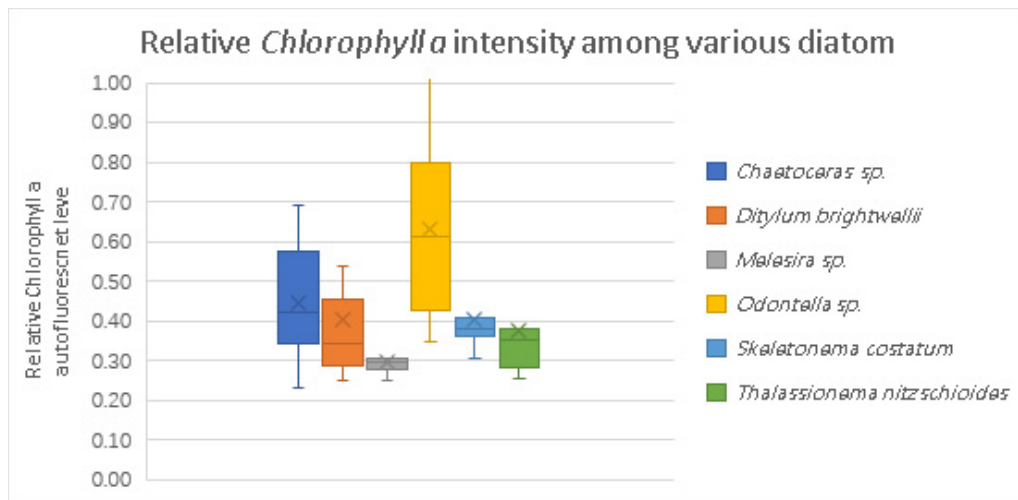


Figure 4. Relative chlorophyll *a* intensity in six dominate diatoms in the examined water samples.

Discussion

In general, a high auto-fluorescence intensity could represent a high concentration of chlorophyll *a* as more PS II will be capable of reflecting the incoming light. A high auto-fluorescence level could also imply a relative low efficiency in photosynthesis as most light providing energy to the chlorophyll for photosynthetic processes was being reflected instead of absorbed. In this study, *Odontella* sp. demonstrated the highest level of fluorescence and therefore the highest level of chlorophyll *a* amount. Such a phenomenon could be explained by the high cell length of *Odontella* sp. (15 – 110 μ m) with numerous chloroplasts (The Phytoplankton Encyclopedia Project, <https://phytoplankton.eoas.ubc.ca/index.html>). On the other hand, *Melosira* sp. examined in this study demonstrated

the lowest level of fluorescence and therefore the lowest level of chlorophyll a amount. It could potentially result from a lower cell size ranging from 11-30 μm (Castenholz, 1964). High fluorescence level of *Odontella* sp. and low fluorescence level of *Melosira* sp. also indicated a corresponding low PS II efficiency and high PS II efficiency. Since *Odontella* sp. has numerous chloroplasts within larger cell size, the chloroplast efficiency would not be high in comparison to *Melosira* sp. experiencing a smaller cell size.

Fluorescence variations were also analyzed. *Odontella* sp. experienced the highest intraspecies fluorescence level variation while *Melosira* sp. experienced the least. Variations within *Odontella* sp. could potentially result from morphological changes occurring within *Odontella* species. Two *Odontella* cells could attach themselves by elevations and thus giving out an overall stronger fluorescence compared to only one cells, differentiations between difference *Odontella* cells could resulted in wide range of fluorescence variation (Lavigne et al. 2015). *Melosira* sp. experienced the least amount of variation as the relative lengths of the species remained constant as approximately 500 μm from the experiment data, thus giving out the least number of variations.

Certain limitations of this study include a small sample size, two-dimensional analysis of algae and environmental variations. The general sample collected for the study was relatively small and scattered. The samples for each diatom species range from 1 to 13 samples. Such variation makes analysis of each species not statistically significant and deviate with the accurate data. Another limitation came from the structure of diatoms. ImageJ processing of sample images only took the surface area of chlorophyll within each diatom for analysis but did not consider the 3D structure of each diatom. Lastly, the collection and analysis of the sample were done in 2 separate trials, containing a 24–48-hour gap. Time interval between the analysis could result in unexpected sample differences, such as changes in internal metabolism. However, the general trend discovered in the finding still held significance and could be used in future applications.

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