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Fluorescence from a single *Symbiodinium* cell

Christine Guzman^{1,2}, Xue Han^{1,4} , Eiichi Shoguchi³ and Sile Nic Chormaic¹

¹ Light-Matter Interactions Unit, Okinawa Institute of Science and Technology Graduate University, Onna, Okinawa 904-0495, Japan

² Evolutionary Neurobiology Unit, Okinawa Institute of Science and Technology Graduate University, Onna, Okinawa 904-0495, Japan

³ Marine Genomics Unit, Okinawa Institute of Science and Technology Graduate University, Onna, Okinawa 904-0495, Japan

E-mail: xue.han@oist.jp

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Abstract

The partnership between coral and its algal symbionts, *Symbiodinium*, is crucial to the global environment. Yet, the regulatory process within the photosynthetic machinery of *Symbiodinium* is still not clearly understood. Here, we studied the influence of light stress from focussed red and blue lasers on single *Symbiodinium* cells. Fluorescence signals were measured to show cell response. Increasing the incident laser power or the exposure time resulted in an increase followed by a decline in fluorescence intensity. The trend of fluorescence intensity changes was associated with mechanisms of light use efficiency, non-photochemical quenching, photoinhibition, and repair of the cell. Our study provides new approaches to studying the photobiology and physiology of *Symbiodinium* cells.

Introduction

Corals are particularly susceptible to elevated seawater temperatures and exhibit bleaching or lose their algal endosymbionts of the genus *Symbiodinium* under stress. *Symbiodinium* belong to phylum Dinoflagellata, which is one of the largest groups of marine phytoplankton. *Symbiodinium* cells perform photosynthesis within the coral host cells. The photosynthetically-produced organic nutrients are translocated to the coral host and contribute substantially to its growth and calcification [1]. In return, the coral host provides a protective environment and a supply of nutritious metabolic waste products to the symbionts. Such partnership between the coral host and its symbionts is known to be highly sensitive to environmental stressors such as high seawater temperature and high solar irradiance [1–3].

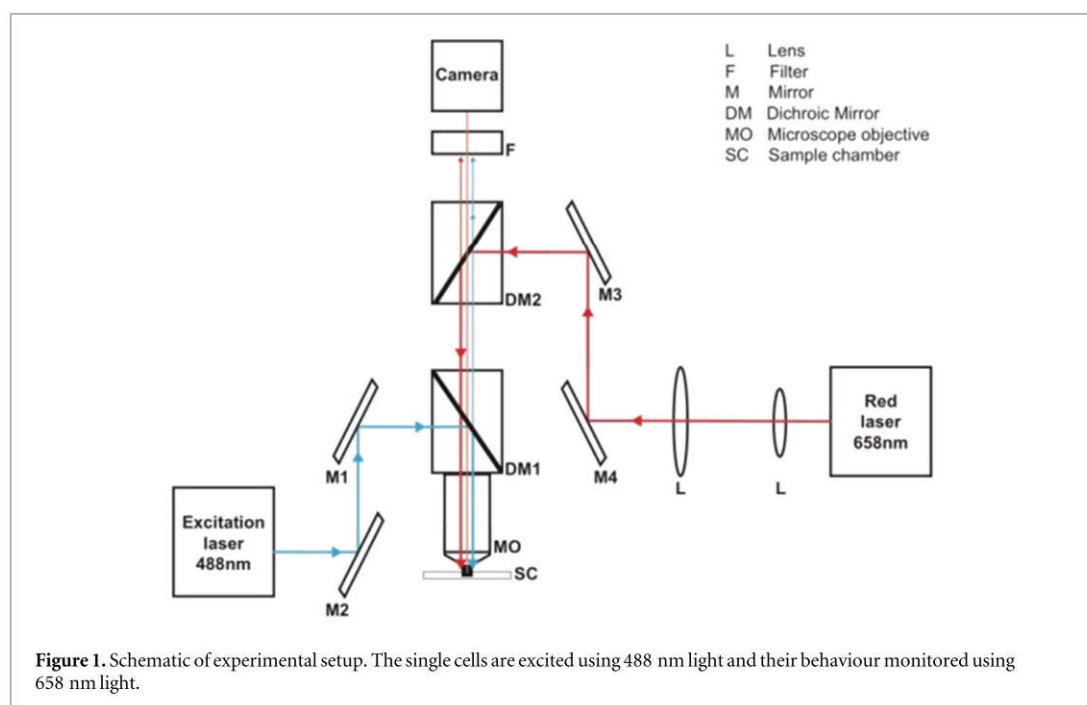
Given the ecological and economic importance of corals, intensive studies have been done in an attempt to understand the photobiology and photophysiology of *Symbiodinium*. The fluorescence emission of chlorophyll *a* (chl *a*), the primary photosynthetic pigment of *Symbiodinium*, is commonly used to evaluate the photosynthetic fitness of the algal symbiont as well as the physiological condition of the coral [4–8]. Chlorophyll fluorescence is the re-emitted light that represents the fate of excitation energy in the photosynthetic apparatus [9].

In most coral studies, non-invasive methods such as pulse-amplitude-modulated (PAM) and fast-repetition-rate (FRR) fluorometers are capable of detecting total fluorescence of cell populations *in vitro* or *in hospite* [10, 11]. Extracted chl *a* pigment has also been extensively studied [4, 5]. However, natural variations in pigment content and thickness of the membrane layer can exist among cells of the same *Symbiodinium* type. So far, conventional fluorometers are not able to provide measurements for single *Symbiodinium* cells [4, 5, 12]. Only a few articles have reported fluorescence intensity studies for single phytoplankton cells [13, 14].

In this study, we performed optical stimulations to single *Symbiodinium* cells using a focussed laser beam. Dependences of the fluorescence intensity on incident laser power and exposure time were examined. Our findings may provide new insights into the photobiology and physiology of a *Symbiodinium* cell under light stress.

Methods

Two strains of cultured *Symbiodinium*, *Symbiodinium* sp. (type A3, cell size = 10–12 μm) and *S. minutum* (type B1, cell size = 6–8 μm), were maintained at 25 °C as previously reported [15]. All cultures were maintained in artificial seawater (Guillard's (F/2)



marine-water enrichment solution, Sigma-Aldrich). Three antibiotics, ampicillin ($100 \mu\text{g ml}^{-1}$), kanamycin ($50 \mu\text{g ml}^{-1}$), and streptomycin ($50 \mu\text{g ml}^{-1}$) were also included. Mass culturing was performed in a 2 litre final volume by inoculating, and sampling was performed once every three weeks. The incubator was maintained on a 12 h-light/12 h-dark regime with illumination.

An optical tweezer (Thorlabs EDU-OT1), containing a red laser ($\lambda = 658 \text{ nm}$), was configured with a blue excitation light source ($\lambda_{\text{ex}} = 488 \text{ nm}$) for fluorescence ($\lambda_{\text{fluor}} \approx 675 \text{ nm}$) experiments. The schematic of the experimental setup is shown in figure 1. Studies have reported that chl *a* pigment is usually located near the cell wall. Thus, the focus spots of both the red and blue laser beams were overlapped and directed near the cell's periphery. The red laser was focussed through a Zeiss 63X, 0.8NA objective. The excitation beam was collimated and directed into the same objective lens through a dichroic mirror. Based on the incident laser beam wavelength (658 nm) and the objective lens, the diameter of the incident beam was approximately $1 \mu\text{m}$ based on the diffraction limit; this is a few times smaller than the average diameter of the *Symbiodinium* cells. A localised spot on the *Symbiodinium* cell was exposed to light stress and excited for fluorescence emission. Sample illumination was accomplished using a white LED, and a CMOS camera (Thorlabs DCC1645C) was used to observe *Symbiodinium* and collect the fluorescence (recording parameters: rate = 83 fs^{-1} , exposure time = 10 s). Laser powers were measured before the microscope objective lens. Time-lapse recordings were cropped using ImageJ with only the cell region included. The fluorescence intensity was obtained using a custom script in

MATLAB. A longpass filter (650 nm) was placed in front of the camera to remove stray light from the red and blue laser beams.

Results

Without red laser influence

To verify if short-term exposure to the blue laser has an effect on the fluorescence measurements, a control measurement without the red laser being present was performed. Fluorescence measurements were carried out at two-minute intervals using only the blue excitation laser (incident power $45 \mu\text{W}$) applied for 10 s, and a video was recorded simultaneously. Two cells per *Symbiodinium* type were studied and the average fluorescence emissions were calculated. Within 10 s of exposure to the blue laser, the intensity of the fluorescence did not change significantly for either *Symbiodinium* type, as shown in figure 2. The fluorescence intensities of types A3 and B1 could be considered as constant under a natural and normal environment, without any red laser beam influence.

With red laser influence

The fluorescence intensity of a single cell was obtained following a two-minute red laser exposure with 10 s of the blue laser excitation. The duration of one series of measurements was approximately 30 minutes. During fluorescence measurements, the red laser was turned off and the excitation blue laser opened for 10 s. The average fluorescence intensities were calculated and plotted against the laser operating current for both *Symbiodinium* types as shown in figure 3. In this figure, laser power is plotted on the right side against laser

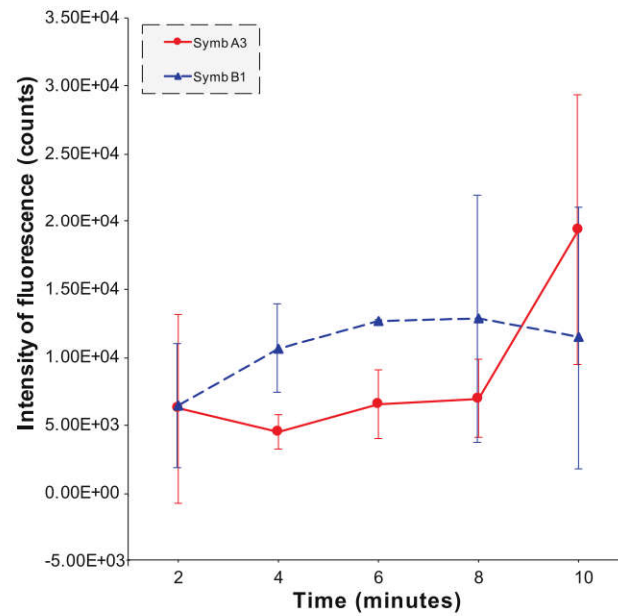


Figure 2. Fluorescence intensities of *Symbiodinium* A3 and B1 cells for ten-second blue laser exposure in two-minute intervals with no red laser influence.

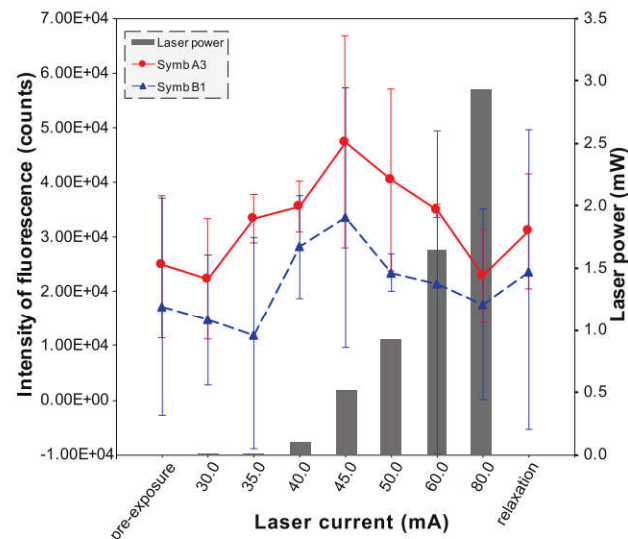


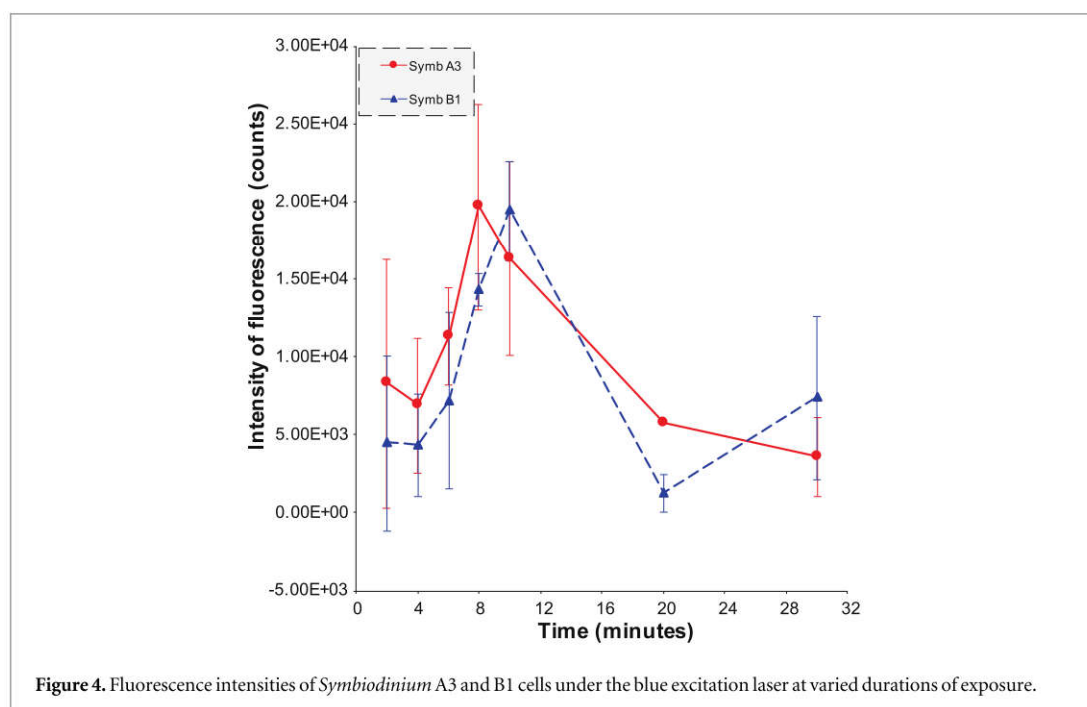
Figure 3. Fluorescence intensities of *Symbiodinium* A3 and B1 cells under two-minute red laser exposure with varied laser currents (y-axis on the left side). Fluorescence intensities for both pre-exposure and after exposure relaxation are also included. The laser power for each laser current is plotted with respect to the y-axis on the right side (grey bars).

operating current. Fluorescence intensity started to increase until 0.5 mW laser power input was applied, after which it decreased gradually with further increasing laser power. Specifically, the fluorescence intensity of type A3 started to increase after 35 mA (threshold current) of red laser. Meanwhile, an increase in type B1 fluorescence was observed at 40 mA exposure. Fluorescence intensities before and after the red laser exposure are also demonstrated. Interestingly, the fluorescence intensity returned to a value similar to pre-exposure levels when the cells were allowed to be

relaxed from the exposure to the red laser beam for five minutes, after the last red laser exposure using the strongest exposure parameters (80 mA laser current, 3 mW laser power).

With blue laser influence

Although exposure to the excitation blue light under a short time (10 s in our study) did not significantly affect cell fluorescence, a study has reported that *Symbiodinium* cells are more sensitive to short light wavelengths (UV/purple region) [4]. Thus, we also



exposed the cells to a blue laser beam continuously at a fixed incident power of $45 \mu\text{W}$ for up to thirty minutes and collected the fluorescence intensity for 10 s at varied time points. Similarly to our results under red laser beam influence, an initial increase in fluorescence was observed in the first 10 minutes and a decrease was observed with longer exposure time, as shown in figure 4. Fluorescence was no longer detectable after 40 minutes. For recovery, a 20-minute interval was provided after which fluorescence could not be observed again. Compared to the control experiment results, a steep slope of fluorescence increase was observed for both types of *Symbiodinium* cells. After reaching the peak, the fluorescence intensity decreased with further increasing the exposure time. Although the incident power from the blue laser was not as strong as the red laser, the effect from light stress was observed.

Discussion

In our study, we performed localised illumination of red and blue lasers on single *Symbiodinium* cells as well as localised fluorescence excitation. The change of fluorescence intensity was measured at the single-cell level. This is in contrast to many studies wherein fluorescence measurements are acquired from extracted pigments solutions, cell cultures, or coral fragments [4–8].

In this study, we compared the light stress responses of *Symbiodinium* cells A3 and B1, both of which have been previously studied under heat stress [16]. Although we observed high variations in chl *a* fluorescence from the same type of cells under the same

excitation conditions, the trend of the fluorescence signal change was apparent for both types. It is widely known that *Symbiodinium* exhibits high genetic and physiological diversity within and among different types [12, 17, 18]. Variations in fluorescence intensity could be attributed to the morphological characters and physiological features of the cells [19], e.g. size of the cell, concentration of pigment, and the thickness of the membrane layer.

We separate the trend of fluorescence change into three phases: (i) increase to a peak, (ii) decrease after the peak, and (iii) recovery after light stress release. In both the red (power dependent) and blue (exposure time dependent) laser experiments, the fluorescence intensity increased initially until it reached a peak. Absorption of photons results in singlet-state excitation of chl *a* which can be relaxed to the ground state by emitting fluorescence [1], hence an increase in fluorescence was observed. With increased exposure time or light power, photosynthetic organisms perform various protective mechanisms, and the relationship among these mechanisms is not well understood so far. One of the protective mechanisms is called non-photochemical quenching (NPQ), which dissipates excessive absorbed light through heat [4, 10, 20]. Because of NPQ, the slope of the fluorescence increase reduced with higher laser power or longer exposure time, and the fluorescence intensity reached a peak.

After reaching the peak, the fluorescence intensity decreased with further increased laser power or exposure time. With further redirection of the light energy via NPQ, the yield of the fluorescence could be decreased [10]. At the same time, impairment of functional Photosystem II (PSII) centres due to absorption of high light energy could also happen [3, 10]. A PSII

centre is composed of a multi-subunit pigment-protein complex that drives a photochemical reaction [21]. Upon light or heat stress, highly reactive intermediates and by-products, such as reactive oxygen species (ROS), will cause photoinhibition and consequently photodamage to the photosynthetic apparatus [3, 4, 7, 10]. However, the PSII is constantly repairing itself from the damage [22, 23]. Therefore, photoinhibition occurs when the rate of damage exceeds the rate of repair [3]. This could be the reason we observed a decrease in fluorescence with increased laser power or exposure time.

We measured the fluorescence after cells were released from the light stress associated with the red or blue laser, to check the recovery of these cells during the repairing process. However, the response to the blue and red lasers was different when the relaxation period was considered. After the cells were released from the red laser beam, the fluorescence intensity could return to the pre-exposure level for both types of *Symbiodinium*. For the blue laser exposure, fluorescence signals were no longer detectable even after long relaxation periods of time, up to 20 min, were given. This implies that complete photodamage of all the PSII reaction centres within the cell may have already occurred. It has been reported that *Symbiodinium* are more sensitive to photodamage under shorter light wavelengths [4].

From our study, both fluorescence increase and decrease was observed in a single *Symbiodinium* cell belonging to type A3 or B1. Temperature change from heating of the water solution via the applied focussed laser beams may not be the primary contributing factor to the changes in fluorescence intensity. In a study that utilized a 1040 nm laser (300 mW) to heat and measure the fluorescence of single marine phytoplankton cells, the temperature increase induced by the laser was 4.0 °C [14]. In our experiments, the maximum red laser power was only 3.0 mW, hence the temperature increase is expected to be lower than 0.04 °C since water has a much lower absorption coefficient ($<100\times$) at 658 nm than at 1064 nm [24, 25].

Conclusion

The fluorescence intensity of single *Symbiodinium* A3 and *Symbiodinium* B1 were studied under two laser beams (488 nm and 658 nm) separately. We detected a fluorescence rise, of about 10 000 counts, from a single cell from its initial stage while increasing the incident laser powers or exposure time. With further increasing incident power or exposure time, a decrease in fluorescence intensity was observed. To our knowledge, non-photochemical quenching and photoinhibition were observed, for the first time, in a single *Symbiodinium* cell. Fluorescence measurement of an intact single cell, rather than cell cultures, cells *in hospite*, or pigment extracts, could improve our

understanding of the photobiology and physiological response of *Symbiodinium*. A more delicate and systematic design of the experiments could also be considered in order to study single *Symbiodinium* cells, e.g. using optical tweezers to suspend a single cell in solution or to trap a single motile cell and examine the fluorescence.

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ORCID iDs

Xue Han  <https://orcid.org/0000-0001-9525-1176>

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