Research Article

Algae 2010, 25(3): 133-140
DOI: 10.4490/algae.2010.25.3.133
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Effect of light and sediment grain size on the vertical migration of benthic diatoms

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Using chlorophyll fluorescence, the vertical migration of benthic diatoms responding to light intensity and affected by sediment grain size was studied. Minimal fluorescence (F_o) of surface sediment was measured by imaging pulse amplitude modulated (Imaging-PAM) fluorometer, and used to monitor diatom biomass variation in surface sediments. The test diatoms, *Amphora coffeaeformis* (C. Agardh) Kützing and *Cylindrotheca closterium* (Ehrenberg) Reimann & Lewin, migrated to the sediment surface under irradiance from 50 to 500 μmol photons m^{-2} s^{-1}. However, the diatoms exhibited no evident increase of surface biomass under dark conditions, and even showed slightly decrease of surface biomass under irradiances over 1,000 μmol photons m^{-2} s^{-1}. The light intensity inducing the maximum surface migration of *A. coffeaeformis* was 100 μmol photons m^{-2} s^{-1}, while the light intensity producing the same effect for *C. closterium* was 250 μmol photons m^{-2} s^{-1}. *C. closterium* showed higher motility than *A. coffeaeformis*. Faster diatom surfacing was observed in larger grain size sediments (125-335 μm) than smaller ones (63-125 μm). This study confirmed the significant influence of light as a main triggering factor behind migration, indicated the distinct effect of different sediment grain size, and highlighted the species-specific migratory ability.

Key Words: benthic diatom; chlorophyll fluorescence; phototaxis; pulse amplitude modulated; vertical migration

INTRODUCTION

Benthic diatoms inhabiting intertidal sediments exhibit vertical migratory rhythms within the upper sediment layers, which are associated with diurnal and tidal cycles (Round and Palmer 1966, Palmer and Round 1967, Joint et al. 1982). This phenomenon is particularly well documented for estuarine intertidal microphytobenthos (MPB)(Guarini et al. 2000, Consalvey et al. 2004). Since vertical migration of microphytobenthos has been largely recognized as a key controlling factor of short-term variability in microphytobenthic productivity, it has been studied increasingly in recent years (Pinckney and Zingmark 1991, Serôdio et al. 2001).

It has been considered that the main reasons behind this vertical migration are endogenous phototaxis and geotaxis, primarily in response to light and tide (Harper 1977, Consalvey et al. 2004). Furthermore, the diel cycle of light is the main factor triggering MPB migration in subtidal zones with nearly no influence of tidal cycles

Received 27 March 2010, **Accepted** 15 August 2010
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(Ní Longphuirt et al. 2006). Due to the large variation of light intensity under *in situ* intertidal conditions, and the direct effects of light on the functioning of the photosynthetic apparatus, migratory response of MPB to light is particularly interesting (Serôdio and Catarino 2000, Serôdio et al. 2006).

Some environmental factors, such as temperature and salinity, affect MPB motility (Paterson 1986, Cohn and Disparti 1994, Sauer et al. 2002, Cohn et al. 2003). Besides, sediments grain size is an important factor that is closely associated with light penetration, porosity, water content, and dissolved nutrients, as well as MPB biomass and species composition (Underwood and Kromkamp 1999, Mitbavkar and Anil 2002, Bale and Kenny 2005). This factor is also thought to influence the speed or depth to which diatom cells migrate (Hay et al. 1993, Consalvey et al. 2004). Previous studies have proven that speeds of diatom movement are different on various substrata, and vertical speeds are an order of magnitude lower than horizontal speeds (Hopkins 1963, Harper 1977, Hay et al. 1993). Migratory speed of diatoms is comparatively slower in sediments than on artificial substrata, such as glass slides. It has been observed in the field that the diatoms concentrated at a depth of 1 mm can migrate up to the surface in 1.5 hours (Hopkins 1963). Sediments components are different in grain size. Therefore, knowing the effect of grain size on migration is helpful for analyzing motility of diatoms in sediments, and for elucidating the distinction of species composition in different sediments.

Various techniques have been utilized to investigate vertical migration, including direct observation of color change (Aleem 1950, Perkins 1960), the lens tissue technique (Eaton and Moss 1966), and cryofixation for low-temperature scanning electron microscopy (Paterson 1986, Janssen et al. 1999). In recent decade, the techniques utilizing spectral reflectance and fluorescence have been widely used to monitor changes in microphytobenthos biomass (Serôdio et al. 1997, Kromkamp et al. 1998, Paterson et al. 1998, Perkins et al. 2001, Honeywill et al. 2002). Serôdio et al. (1997) initially employed fluorescence techniques that use a pulse amplitude modulated (PAM) fluorometer to monitor biomass of benthic microalgae. It showed that minimum fluorescence (F_o) is less sensitive to temperature and irradiance fluctuations compared to other fluorescence variables, and has a linear relationship with microphytobenthic biomass (Serôdio et al. 1997, 2001, Barranguet and Kromkamp 2000, Honeywill et al. 2002). Furthermore, Imaging-PAM has more advantages than other types of PAMs. Because it can measure larger surface areas than other PAMs, and define numbers of interesting points simultaneously on one image. Therefore, this technique can decreases experimental error caused by the prolonged time required for measuring samples individually.

Until now, no study has used Imaging-PAM to investigate vertical migratory behavior of benthic diatoms, even though several laboratory studies have examined this behavior by focusing on the influence of light, temperature, and salinity. In this study, we use Imaging-PAM to monitor surface biomass variation of thin layer sediment, which covers the artificial diatom biofilm in the wells of 24-well plates.

We aim to investigate the effects of light and sediment grain size on vertical migration of individual diatom species, as well as analyze the migration mechanism through physiological and morphological characteristics of diatoms with a miniaturized experimental setup.

MATERIALS AND METHODS

Culture of diatoms

Diatoms *Amphora coffeaeformis* (Agardh) Kützing (B-95) and *Cylindrotheca closterium* (Ehrenber) Lewin (B-62), supplied by the Korea Marine Microalgae Culture Center (Busan, South Korea), were used as experiment species for their distinct cell shape. The diatoms were cultured in 2 L flask with f/2 medium and kept at 20°C and 12 h daily illumination with 100 μmol photon m^{-2} s^{-1} of fluorescent light. The growth rates were monitored through increases in chlorophyll *a* concentration and cell number. Prior to experiments, and once the culture reached a constant cell number, diatom cells were harvested by centrifugation (1,000 × g, 5 min).

Treatment of sediment

The sediment, which was collected from the sand flats of Nakdong River estuary, was treated to remove organic materials. Firstly, sediment was sifted to remove shell fragments and gravel. Then, it was rinsed several times with tap water to remove most of the salt. Subsequently, 33% H_2O_2 was added to the sediment. It was mixed and left for several days to allow for the complete reaction to remove organic matters (Taylor et al. 2005). After decanting the overlying water, sediment was rinsed with distilled water at least 10 times, and then with deionized water at least 5 more times. Finally, sediment was dried

at 60°C in a dry oven for 8 h. Certain sediment of specific grain sizes (63-125, 125-250 and 250-335 μm) was obtained by serially dry-sieving.

Sediment chlorophyll *a* concentration and minimal fluorescence (F_o)

This experiment determined variation of F_o with sediment chlorophyll *a* concentration in samples prepared from treated sediments to which diatoms were added. Harvested diatoms were increasingly diluted with f/2 medium to obtain a large range of chlorophyll *a* concentration. Each 2.0 mL diluted diatom sample was mixed well with identical sediment volumes (approximately 0.5 g). Due to the well depth influence on the imaging of samples in the marginal wells, 3.0 g of treated sediment (125-250 μm) was added to every well as a base to adjust the sample height. Before each sample was added, a piece of filter paper (2.3 μm glass fiber) was placed to separate the base and the sample. Subsequently, each sample was added over the filter paper. All the sediments in wells were thoroughly saturated with fresh f/2 medium.

During every measurement using the Imaging-PAM fluorometer (Max/L, Walz, Germany), the well plate was put on a fixed mounting stand position under the measuring head of the Imaging-PAM. Before measuring fluorescence, areas of interest (AOIs) were defined under Live Video Mode, with the same size as a plate well. The same AOIs were consistently used in one set of the experiment. After a 5 min dark adaptation, F_o of samples was measured. The fluorescence was induced by royal blue (450 nm) 3 W Luxeon LEDs, which have standard intensity of 0.5 μmol m^{-2} s^{-1} and modulation frequency between 1 and 8 Hz. One fluorescence image was shown as an example in Appendix Fig. S1. The fluorescence values were exported as Microsoft Excel data.

After measuring fluorescence, the chlorophyll *a* of sediments over filter paper was extracted in 90% acetone at 4°C under dark conditions. Chlorophyll *a* concentration was measured according to Lorenzen's (1967) method by spectrometer (Agilent 8453; Agilent Technologies Inc., Santa Clara, CA, USA). The tested samples for two species, *A. coffeaeformis* and *C. closterium*, were 11 and 9, respectively.

Experiments on vertical migratory photoresponse

Preparation of the well plate was the same as previously described, viz. adjusting sample height in wells with 3.0 g treated sediments (125-250 μm). Cultured diatoms were deposited homogenously on glass microfiber filters (porosity 2.3 μm) by slow filtration (< 0.1 MPa), and then covered with approximately 1 mm thick sediments. The F_o measured by Imaging-PAM was used to monitor diatom migration from the filter surface up to the sediment surface.

In every experiment set, initial F_o was measured before the filter with diatoms was covered by sediment. After covering with approximately 1 mm thick sediments, F_o was measured at certain time intervals after a 5 min dark adaptation. All wells with saturated samples were maintained during the experiment process.

The first set of experiments focused on the effect of light intensities on the vertical migratory response. Three replicates of each species were treated with 7 light intensities of 0, 50, 100, 250, 500, 1,000, 1,400 μmol photons m^{-2} s^{-1}, respectively. Covered sediments used 125-250 μm grain size sand. Samples were incubated at 20°C. F_o was measured at 0 h, 2 h and 4 h.

The second set of experiments focused on the effect of sediment grain size on the upward vertical migration. Three kinds of grain size sediment (63-125, 125-250 and 250-335 μm) were used to cover the artificial biofilm of three replicates. Samples were incubated at 20°C and 100 μmol photons m^{-2} s^{-1} for up to 2 h. F_o was measured at 0 h, 1 h and 2 h.

Statistical analysis

Univariate analyses, followed by post-hoc Tukey tests, were carried out to test the difference between different light intensities and different grain size sediment using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Correlation of chlorophyll *a* concentration and F_o

Sediment chlorophyll *a* concentration had a significantly linear correlation with F_o (r^2 = 0.9209, $p < 0.001$, Fig. 1). This showed that F_o could be used as a proxy to indicate biomass variation at sediment surface. Correlation index (r^2) was slightly higher than pooling of all data, but there were no differences in the slopes of the two species ($p > 0.05$), regarding to the linear equations of individual species (Fig. 1).

Effect of light intensity

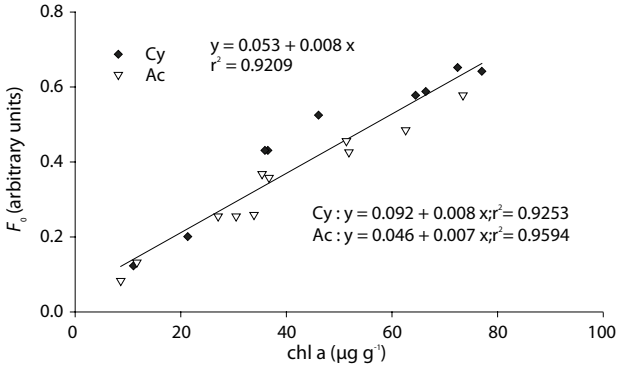

Fig. 1. The correlation of chlorophyll a concentration and F_o. Ac and Cy represent *Amphora coffeaeformis* and *Cylindrotheca closterium*, respectively.

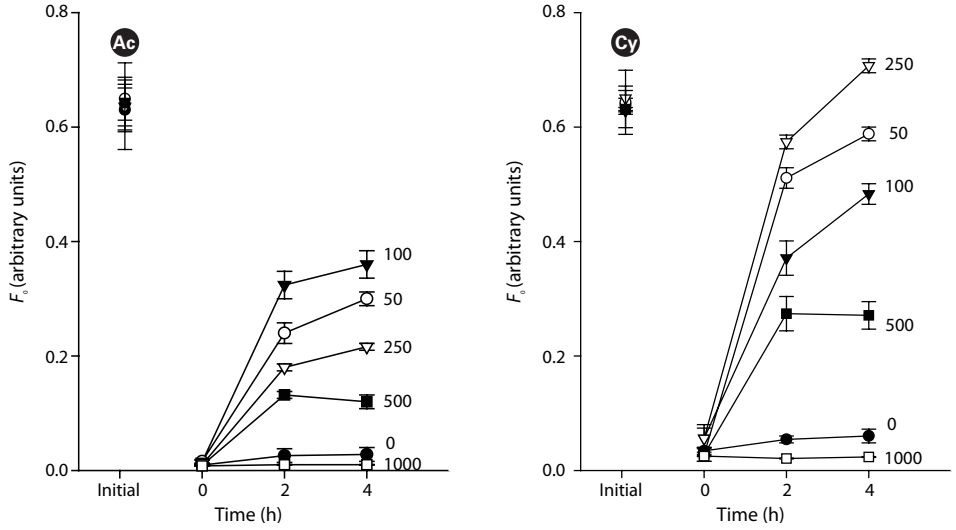

Fig. 2. Effect of light intensity on the upward migration of individual species Ac: *Amphora coffeaeformis* and Cy: *Cylindrotheca closterium*. Error bars indicate standard deviation of triplicates. Uints of light intensities 0, 50, 250, 500, 1,000 are μmol photons m^{-2} s^{-1}. The initial on x-axes is measurement time before covering the diatoms biofilms with sands.

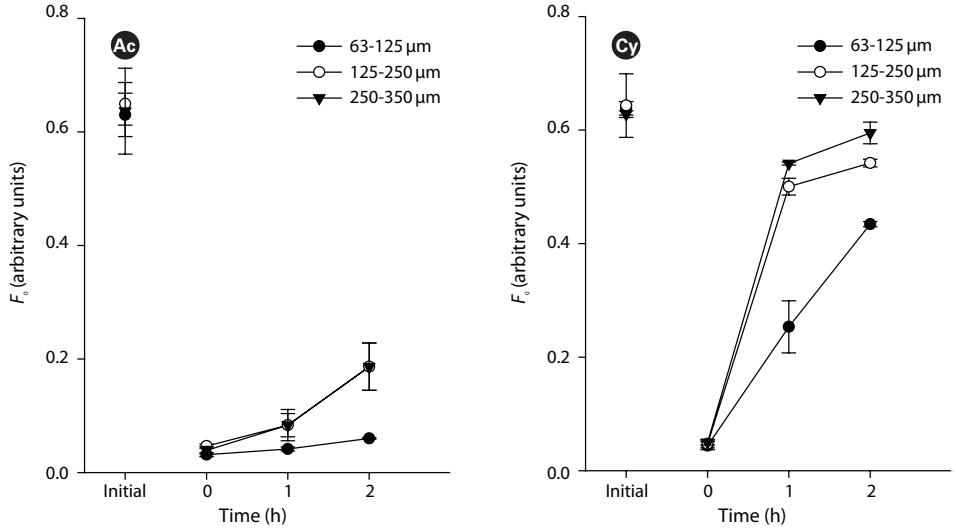

Fig. 3. Effects of grain size on the upward migration of individual species Ac: *Amphora coffeaeformis* and Cy: *Cylindrotheca closterium*. Error bars indicate standard deviation of triplicates. The initial on x-axes is measurement time before covering the diatoms biofilms with sands.

Fig. 2 illustrates the migratory responses of individual species to different light intensities. The lowest F_o existed under 1,000 and 1,400 μmol photons m^{-2} s^{-1} (not shown) which equaled that of blank samples (0.002-0.0025 arbitrary unit) and was even lower than under dark conditions. Under 500 μmol photons m^{-2} s^{-1}, both species showed decreased surface biomass after 4 h of illumination. The light intensity inducing the maximum surface migration for *A. coffeaeformis* was 100 μmol photons m^{-2} s^{-1}, while *C. closterium* was 250 μmol photons m^{-2} s^{-1}. Univariate analysis following the post-hoc Tukey tests showed there was no significant difference between 0 and 1,000 μmol photons m^{-2} s^{-1} for either species ($p > 0.05$). After 4 h of illumination, there was no significant difference in the effect on either species among 50, 100 and 250 μmol photons m^{-2} s^{-1} light intensity ($p > 0.05$). The difference of 500 μmol photons m^{-2} s^{-1} with 50, 100 and 250 μmol photons m^{-2} s^{-1} was significant for *C. closterium* ($p < 0.05$). However, for *A. coffeaeformis*, the difference between 250 and 500 μmol photons m^{-2} s^{-1} was not significant ($p > 0.05$, Fig. 2).

Effect of grain size

Small (63-125 μm) grain size showed significant difference from medium (125-250 μm) and large (250-335 μm) grain size ($p < 0.05$, Fig. 3). However, there was no difference between middle and large grain sizes ($p > 0.05$, Fig. 3). Comparing similar initial values, *C. closterium* showed evidently higher motility than *A. coffeaeformis*, which surfaced up to 60% of initial biomass on the artificial biofilm within the first 1 h.

DISCUSSION

Validity of laboratory studies

The designed experimental setting in this study was proven valid, practical and convenient for studying vertical migratory behaviors of benthic diatoms. Despite an unavoidable oversimplification of natural variability, a laboratory-based investigation was still appropriate for studying the effects of some main environmental factors on vertical migration. Under the experimental conditions in this study, it was evident that cultured diatoms showed their phototaxis by moving towards the light. The stratified structure used in the experimental design was representative for benthic biofilms *in situ*, and was convenient and feasible for monitoring vertical migration. Méléder et al. (2003) also employed a filtering method that allowed diatom cells to uniformly deposit on microfiber filters for the reflectance measurement of monospecific diatom cultures. Additionally, the filtering method showed no obvious damage to cells by scanning electron microscopy. Furthermore, a 24-well plate with Imaging-PAM allowed the synchronous and rapid measuring of a number of samples with less influence on the experiment treatment.

Monitoring benthic diatoms biomass through minimal fluorescence (F_o)

By measuring minimum fluorescence F_o, PAM fluorometry allows a rapid, sensitive and non-destructive monitoring of variations in surface microphytobenthic biomass. In this study, the expected linear relationship of F_o and the sediment chlorophyll *a* concentration were obtained and consistent with previous studies (Serôdio et al. 1997, Honeywill et al. 2002, Kromkamp et al. 2006). F_o has shown the least variation in different communities (Serôdio et al. 2001), which can be corroborated by no observed differences between the two species in the slope of the linear relationships in this study. However, in the field, sampling depths for chlorophyll *a* measurement are usually more than 1 mm and cannot be as precise as the μm level unless they are cryo-cut by microtome. Given that the measuring depth of PAM fluorometer was 100 to 200 μm, F_o could only stand for the very surface biomass of 0-100 or 0-200 μm sediments as diatom distribution or movement in deeper sediments could not be detected. Although F_o is still a good indicator for monitoring variation of surface biomass, a vertical scale mismatch may exist between it and chlorophyll *a* concentration of sediment in practical sampling depths (Barranguet and Kromkamp 2000).

A dark adaptation period of 15 min has been suggested to measure F_o (Honeywill et al. 2002, Consalvey et al. 2004, Kromkamp et al. 2006), even though it was thought to be insufficient for complete reversal of non-photochemical quenching (Honeywill et al. 2002). However, due to downwards migration, a dark adaptation period of over 2 min would cause changes in biomass (Serôdio et al. 2006). Also, significantly lower F_o was observed for migratory biofilms after a 5 min dark adaptation as compared to non-migratory biofilms (Jesus et al. 2006a). However, studies have shown that, for migratory biofilms, F_o does not vary significantly between 10 s, 5 min, 10 min, and 15 min dark adaptation (Jesus et al. 2006b). Short periods of dark adaptation, such as 2 min (Serôdio et al. 2006)

and 5 min (Serôdio et al. 1997, 2001), have also been used. Therefore, with known light histories, this study used a 5 min dark adaptation, and it showed no influence on the results.

Light effect on vertical migration

The study revealed obvious phototaxis of diatoms under low to moderate irradiances (50-500 μmol photons m^{-2} s^{-1}). Under dark conditions, without light, there were no evident surfacing movements. High light intensity over 1,000 μmol photons m^{-2} s^{-1} kept the diatoms out of the very surface of sediments where F_o could be measured by PAM. This implies that diatom cells can sense penetrated light intensity and light direction under the sediment. An additional implication is that diatoms can adjust their position through migration to avoid irradiance that is too strong and obtain optimum light intensity for photosynthesis. In regard to the decreased surface biomass after 4 h of illumination under 500 μmol photons m^{-2} s^{-1}, we supposed that diatoms would leave the very surface sediment after enough photosynthesis, or as a result of increasing photoinhibition during prolonged high-light treatment.

Vertical migratory response to different irradiances has also been observed on intact biofilms of estuarine sediments (Serôdio et al. 2006), namely that surface biomass increases under irradiances below 100 μmol photons m^{-2} s^{-1}, reaches maximum under 100-250 μmol photons m^{-2} s^{-1}, and gradually decreases under higher irradiances.

Grain size effect on vertical migration

Light penetration depth in sediment is closely related with sediment characteristics. Penetration depth in reconstituted and intact sediment was 2-3 mm at most, and deeper in larger size sediment under higher light intensity (MacIntyre et al. 1996). Sediment porosity (i.e., the space that diatoms move through between the grains) is closely related to sediment compaction and grain size (Flemming and Delafontaine 2000). However, the influence of sediment fabric, bulk density, and porosity on the speed of diatom locomotion through sediments has remained unclear until now.

This study is the first attempt to determine the effect of sediment grain size on vertical migration of different species. The obvious difference between small grain size sediment and medium and large grain size sediment is the effect on the upwards migratory photoresponse. It confirmes that sediment characteristic are important factors in influencing the diatoms migration. One reason is that diatom cells can easily sense stronger light stimuli, inducing upward migration in sediment with larger grain size. Another important reason is that larger size sediment grains supply a larger space and shorter traveling distance for the movement of diatom cells. In other words, under experimental conditions without disturbance by hydrodynamic forces, grain size effect on diatom migration is mostly related to its physical property of porosity and surface area.

Species-specific migratory response and ability

In this study, migratory response to light was different between two species. The diatom *C. closterium* showed a maximum surface migration under 250 μmol photons m^{-2} s^{-1}, which was higher than that of *A. coffeaeformis* (100 μmol photons m^{-2} s^{-1}). This species-specific variation in migration has already been found in field investigations, which different species migrated to sediment surface at different time during a day following varied irradiance (Paterson 1986, Hay et al. 1993, Underwood et al. 2005, Serôdio et al. 2006).

These species-specific responses have their origin in physiological characteristics. Round and Palmer (1966) observed that *Pleurosigma angulatum*, which is dominant in diatom biofilms at midday, had a higher E_k (minimum saturating irradiance) between 500 and 600 μmol photons m^{-2} s^{-1}, while *Nitzschia dubia*, which displays rapid vertical migration away from the surface with increasing irradiance, has an E_k of 300 μmol photons m^{-2} s^{-1}. The E_k of our two cultured diatoms, *C. closterium* and *A. Coffeaeformis*, were 149 and 113 μmol photons m^{-2} s^{-1}, respectively (data not shown). The slightly higher E_k of *C. closterium* may determine its higher irradiance (250 μmol photons m^{-2} s^{-1}) inducing maximum surfacing biomass.

Besides, higher motility of *C. closterium* was proven by its quicker migration than *A. coffeaeformis*. Comparing cell shape and size, *C. closterium* has long, narrow and only lightly or partially silicified valves (approximately 110 × 5 μm), while *A. coffeaeformis* has a hemispherical shape and nearly semi-circular valves in the lateral view (approximately 20 × 5 μm). Consequently, cells of *C. closterium* can move more quickly through sediment by rotating their frustules than *A. coffeaeformis*, which slide relatively slowly with their bulky bodies.

ACKNOWLEDGEMENTS

This study was supported by the Korean Ministry of Land, Transport and Maritime Affairs as "Greenhouse Gas Emissions Reduction using Seaweeds."

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Appendix

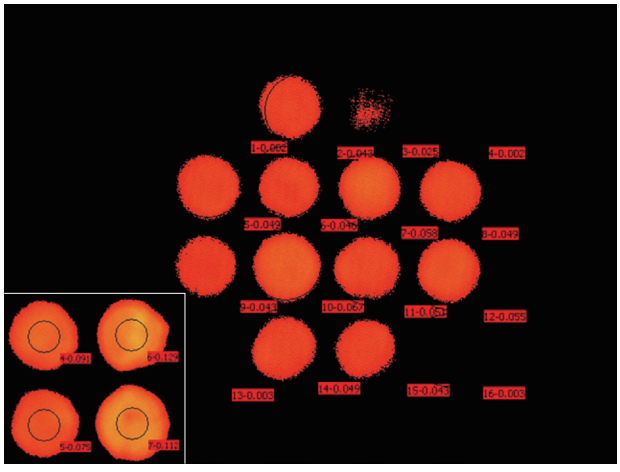

Fig. S1. Fluorescent images taken by Imaging-PAM fluorometer. The areas of interest (AOIs) are the same size as the well of a plate to enclose one whole sample area. The numbers of AOIs are displayed at the lower right of samples with fluorescence values. The well-marked AOIs (black circles) are shown as smaller in size in the left-lower image, which cut from test imaging before practical experiment.