

Rapid Algal Culture Diagnostics for Open Ponds Using Multispectral Image Analysis

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This article presents a multispectral image analysis approach for probing the spectral backscattered irradiance from algal cultures. It was demonstrated how this spectral information can be used to measure algal biomass concentration, detect invasive species, and monitor culture health in real time. To accomplish this, a conventional RGB camera was used as a three band photodetector for imaging cultures of the green alga Chlorella sp. and the cyanobacterium Anabaena variabilis. A novel floating reference platform was placed in the culture, which enhanced the sensitivity of image color intensity to biomass concentration. Correlations were generated between the RGB color vector of culture images and the biomass concentrations for monocultures of each strain. These correlations predicted the biomass concentrations of independently prepared cultures with average errors of 22 and 14%, respectively. Moreover, the difference in spectral signatures between the two strains was exploited to detect the invasion of Chlorella sp. cultures by A. variabilis. Invasion was successfully detected for A. variabilis to Chlorella sp. mass ratios as small as 0.08. Finally, a method was presented for using multispectral imaging to detect thermal stress in A. variabilis. These methods can be extended to field applications to provide delay free process control feedback for efficient operation of large scale algae cultivation systems. © 2013 American Institute of Chemical Engineers Biotechnol. Prog., 000:000–000, 2013
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Introduction

Microalgae cultivation is of commercial interest for production of biofuel feedstocks, nutritional supplements, and agricultural feed.^{1–4} Moreover, microalgae can grow using waste carbon dioxide and nutrients from wastewater streams, enabling an added benefit of environmental pollutant mitigation.^{5,6} Efficient and cost effective algae cultivation requires real time algal biomass quantification, culture health monitoring, and biological contaminant detection.^{3,7} However, conventional methods for accomplishing these tasks require culture sampling, large analysis times, and use of sophisticated equipment, which make the feedback highly localized, prone to large lag times, and expensive.⁸

Conventional methods for culture diagnostics

The algal biomass concentration of open ponds, typically about 0.5 g/L,⁷ is usually quantified either by direct biomass weighing of a culture sample⁹ or by measuring a proxy for biomass, typically optical density.⁸ The biomass concentration is then recovered from the optical density using previously generated correlations. These methods are accurate, but require expensive equipment and incur large lag times, precluding the possibility of real time productivity monitoring.

Additionally, continuous culture health monitoring enables preemptive identification of culture crashes. The instantaneous photosynthetic rate of a culture can be measured electrochemically by measuring the dissolved oxygen concentration¹⁰ or fluorometrically with a pulse-amplitude-modulated (PAM) fluorometer.^{11–13} Dissolved oxygen electrodes can be inexpensively deployed into algal cultures, but they only measure the dissolved oxygen concentration. Additional information is needed to recover the oxygen production rate, which is proportional to the photosynthetic rate.¹⁴ On the other hand, PAM fluorometry can be used to measure the quantum yield of the photosynthetic light reactions in a culture,^{11–13} which is a relative indicator of photosynthetic health. However, due to the high cost of PAM fluorometers, a proxy parameter for quantum yield is sought.

Identification of invasive species in algal cultures is generally accomplished by microscopy¹⁵ or molecular analysis.³ Light microscopy provides a straightforward method for identifying contaminants as it requires only a microscope and the ability to visually identify contaminants. Through molecular analysis, DNA fragments specific to known invasive species are amplified and identified using polymerase chain reaction and gel electrophoresis. Both of these methods require expensive equipment and cannot be performed without removing a sample from the culture.

Multispectral image analysis for rapid and large scale culture diagnostics

Multispectral image analysis is an optical diagnostic method that relies on the reflected and backscattered spectral

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radiation from the system being monitored. Any phenomenon that affects these reflected and backscattered radiations can in principle be detected and quantified by this technique. Thus, multispectral imaging provides a highly sensitive and versatile method for monitoring and diagnosing culture productivity, purity, and health. For example, multispectral imaging has been used to monitor biomass concentration in marine environments for much of the last three decades.^{16–19} Typically, satellite imaging equipment is used to measure the “water-leaving radiance” at wavelengths of 440 nm (blue) and at 555 nm (green), with about 10 nm wide bandwidths.¹⁶ The concentration of chlorophyll a has been shown to be inversely related to the ratio of blue light to green light leaving the water due to selective absorption of blue photons by chlorophyll a. More recently, correlations have been developed between the blue to green ratio and the particulate organic carbon (POC) concentration of marine environments, as POC is of greater interest than chlorophyll concentration in biogeochemical cycles.¹⁷

Moreover, some recent studies have used imaging techniques to quantify biomass concentration in algae cultivation systems.^{20–22} We previously reported a method for monitoring the biomass concentrations of algal cultures using a conventional RGB camera.²¹ The red, green, and blue values decreased exponentially with increasing biomass concentration, which enabled correlations to be developed between the color intensities of the culture and the biomass concentration. However, the color intensities were relatively insensitive to biomass concentration at concentrations typically used in large scale cultivation systems, limiting the applicability of the technique.

Additionally, remote multispectral image analysis has also been employed for identifying stress and invasive species contamination in higher plants.^{23–25} Carter investigated the relationships between eight different types of plant stress and spectral reflectance in six types of vascular plants.²³ The author found that the total reflectance in the visible region increased with most types of stress, and that the increase in reflectance was most pronounced in the green region. To the best of our knowledge, multispectral imaging has not been used for identifying stress or invasive species presence in algae cultivation systems.

In the current study, a multispectral imaging approach is presented for monitoring the biomass concentration, culture health, and invasive species concentration of algae cultivation systems. This approach makes use of a novel floating reference platform, which enhances the sensitivity of the culture image color to its radioactive properties compared to methods previously reported.²¹ Moreover, to minimize equipment costs, this study investigates the utility of an RGB camera in lieu of more expensive hyperspectral imaging equipment. This method can provide delay-free culture diagnostics to inform decisions regarding biomass harvesting, nutrient delivery, and pH regulation in real time.

Materials and Methods

Culture selection and batch cultivation

The green alga *Chlorella* sp. (UTEX 2168) and the cyanobacterium *Anabaena variabilis* (ATCC 29413-U) were used in this study. *Chlorella* sp. is a spherical green alga approximately 5 μm in diameter. It contains the pigments chlorophyll a, with absorption peaks at 440 nm and 680 nm,

chlorophyll b, with peaks at 660 and 480 nm, and carotenoids, with an absorption band between 400 and 500 nm.⁸ *Chlorella* sp. is of interest in the biofuels market due to its high lipid productivity,¹ as well as in the health food market due to its richness in protein, vitamins, polysaccharides, polyunsaturated fatty acids, and microelements.^{2,3}

A. variabilis is a cyanobacterium composed of cells of approximately 5 μm in diameter forming filaments more than 100 μm long.²⁶ It has been used ubiquitously in experimental studies on photobiological CO₂ mitigation and biohydrogen production.^{27–29} Similarly to *Chlorella* sp., *A. variabilis* contains chlorophyll a and carotenoids, but it does not contain chlorophyll b.³⁰ Additionally, *A. variabilis* contains the phycobiliproteins phycoerythrin, phycocyanin, and allophycocyanin, which have absorption peaks at 565, 620, and 650 nm, respectively.³⁰ As a result of the difference in pigment content between *Chlorella* sp. and *A. variabilis*, monocultures of these strains appear different to the naked eye, with the former having a green-yellow color and the latter having more of a blue-green appearance.

Each strain was cultivated as a batch monoculture in the BG11 nutrient medium.⁸ For this, 2 liter bottles of BG11 medium were inoculated with 20 ml of a mature planktonic culture which had a concentration of 0.8 g/L. The bottles were sparged with air-containing 2% by volume carbon dioxide at a rate of 400 ml/min, and continuously illuminated with $16 \pm 2 \text{ W/m}^2$ irradiation ($74 \pm 8 \mu\text{E/m}^2\text{-s}$) in the photosynthetically active region (PAR) using cool white fluorescent bulbs (Philips, F32T8). The culture temperature was $27 \pm 3^\circ\text{C}$ throughout the batch cultivation period. Three different batch cultures were used for the biomass quantification experiments, named Cultures 1, 2, and 3. At the time when Culture 1 was used for experiments, 20 ml of Culture 1 was used to inoculate Culture 2, which was in turn used to inoculate Culture 3. Experiments were performed during the exponential growth phase during the second week after inoculation. It was verified that organism pigmentation was independent of culture age in this age range.²¹

Preparing cultures for imaging

An acrylic box measuring 10.0 cm long by 6.4 cm wide by 8.1 cm high was constructed to mimic an open pond cultivation system. First, the box was filled with 480 ml of BG11 medium. Meanwhile, a dense culture was prepared by centrifuging the batch cultures for 5 min at 3000 RPM. The biomass concentration of the dense culture was measured using the dry biomass method. Cultures of known biomass concentrations of *Chlorella* sp. and *A. variabilis* were prepared by adding incremental volumes of the dense cultures to the box, stirring, and removing an equal volume of diluted culture to ensure similar liquid levels in the acrylic box. The top of the box was open and was illuminated with cool white fluorescent lamps (Philips, 4100 K), which provided diffuse irradiation at an irradiance of $3.8 \pm 0.1 \mu\text{E/m}^2\text{-s}$ and negligible collimated radiation.

Imaging platform

Due to absorption of visible light by the organisms, the red, green, and blue intensities of a culture image decrease with increasing biomass concentration.²¹ However, the optical thickness in the visible range of most algal ponds, defined as the product of the extinction coefficient and the

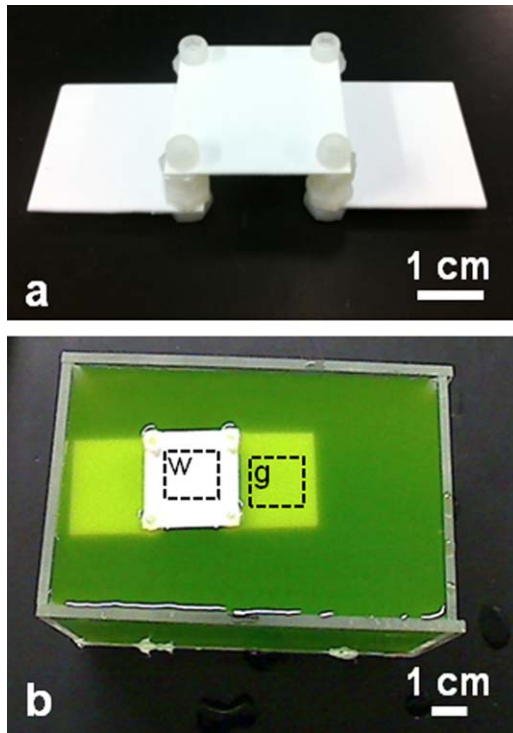


Figure 1. (a) Reference platform used for imaging the cultures and (b) the reference platform in a culture of *Chlorella* sp. at a biomass concentration of 0.4 g/L. The “w” region indicates the white reference region and the “g” region indicates the submerged green region.

physical thickness of the pond, is on the order of 100.⁷ As a result, a saturation effect occurs in the water-leaving radiance and cultures with biomass concentrations greater than about 0.1 g/L are indistinguishable from each other.²¹ In response to this challenge, we constructed a custom reference platform, shown in Figure 1a. When placed in an aqueous medium, the top plate rests above the surface of the water, whereas the bottom plate resides below the surface. This strategy increases the sensitivity of the water-leaving radiance to the micro-organism concentration in the range of concentrations typically employed in algae cultivation systems (0–0.5 g/L). Moreover, normalizing the color intensities from the submerged region with the color intensities of the top region makes the method insensitive to the intensity and spectral content of the light source used to illuminate the culture.

In this study, the top plate of the reference platform consisted of a white square polystyrene sheet 25 mm on a side and 0.5 mm thick (Midwest Products Co., 701-02). The top plate was joined to two identical bottom plates using nylon nuts and bolts (McMaster-Carr, 94812A112 and 95868A260). The vertical distance between the top plate and the two bottom plates was 10 mm. A piece of styrofoam 12 mm long, 6 mm wide, and 2 mm thick was glued onto the bottom side of the top plate to make the platform buoyant. When placed into an aqueous medium, the top plate resided above the liquid surface whereas the two bottom plates resided 10 ± 0.5 mm below the liquid surface, as shown in Figure 1b.

Image analysis

Cultures were imaged using an RGB webcam (Logitech, Pro 9000) within 20 min of preparing the cultures to avoid

changes in pigmentation. A custom computer code was developed to analyze the images. Each pixel of a digital image acquired by the camera is represented by the color vector \vec{c}_p equal to $[r_p, g_p, b_p]$, corresponding to the pixel's red (560–700 nm), green (490–590 nm), and blue (410–500 nm) intensities, respectively.³² Each element of the vector $[r_p, g_p, b_p]$ has an integer value between 0 and 255, inclusive. First, a region of the image containing the top plate above the liquid surface was identified, labeled “w” in Figure 1b. The color vector of this white region \vec{c}_w equal to $[r_w, g_w, b_w]$ was calculated as the average of the red, green, and blue intensities of all the pixels in the region. Then, a region of the image containing a submerged plate was identified, labeled “g” in Figure 1b. The raw color vector of this green region \vec{c}_g equal to $[r_g, g_g, b_g]$ was calculated as the average of the red, green, and blue intensities of all the pixels in the region. To minimize the effects of transmitted light from the side of the chamber, the submerged plate with the greater distance to the nearest wall was used for analysis. The elements of color vector \vec{c} equal to $[r, g, b]$ were calculated by dividing the color intensities of the immersed plate by the color intensities of the top plate to account for differences in the intensity and spectral content of the light source:

$$\vec{c}(i) = \vec{c}_g(i) / \vec{c}_w(i) \quad \text{for } i = 1, 2, 3 \quad (1)$$

Photosynthetic yield

The quantum yield of Photosystem II (YII) is the fraction of the light incident onto the photosynthetic membrane that is converted to short term chemical energy carriers.^{11,12} In this study, YII was measured using a pulse-amplitude-modulated (PAM) fluorometer (Walz, JUNIOR-PAM). The photosynthetic yield was calculated as,^{11,12}

$$Y(II) = \frac{F_m - F_o}{F_m} = \frac{F_v}{F_m} \quad (2)$$

where F_o is the baseline fluorescence from the culture and F_m is its maximum fluorescence after being exposed to a saturating pulse. The difference $F_m - F_o$ is known as the variable fluorescence F_v . The Y(II) value was used as a semi-quantitative indicator of the health of the cells.^{12,33}

Culture invasion simulation

Invasion of cyanobacterial species is often a problem facing large scale green algae cultivation.³ In this study, culture invasion was simulated by adding a known amount of *A. variabilis* to an initially mono culture of *Chlorella* sp. at a concentration of 0.16 g/L. The invasion ratio was defined as the ratio of the mass of *A. variabilis* to the mass of *Chlorella* sp.

Culture crash simulation

Images of an initially healthy culture of *A. variabilis* were continuously acquired as the culture was heated from 23 to 53°C at a rate of $0.3 \pm 0.03^\circ\text{C}/\text{min}$. A hot plate (Fisher, Iso-temp) was used to heat and mix the culture, and a Type T thermocouple (Omega, TT-T-36-100) was used to measure the culture temperature. The maximum photosystem II fluorescence F_m and baseline fluorescence F_o were simultaneously monitored.

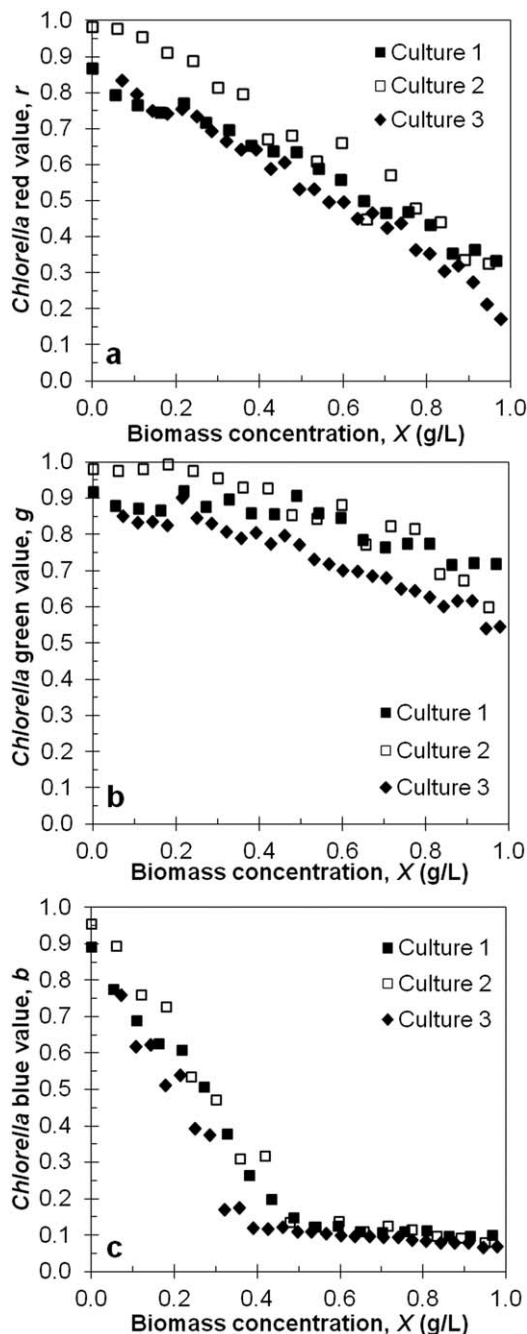


Figure 2. (a) Red, (b) green, and (c) blue values as a function of *Chlorella* sp. biomass concentration.

Results and Discussion

Image analysis of monocultures for biomass concentration quantification

Chlorella sp. Figure 2 shows the red, green, and blue values as a function of *Chlorella* biomass concentration, X , for three different monocultures. When using a culture image as a proxy for measuring its biomass concentration, the color value that is most sensitive to biomass concentration should be used. In the biomass concentration range of 0–0.37 g/L, the blue value was the most sensitive to biomass concentration. Therefore, a least squares regression line was fitted to the experimental data within this concentration range, resulting in the correlation, $X = 0.477 - 0.522 b$, with a coefficient of determination R^2 value of 0.920. At biomass concentra-

tions greater than 0.37 g/L, the blue value was insensitive to biomass concentration due to saturation. Thus, for biomass concentrations between 0.37 and 1.0 g/L, a least squares line between the biomass concentration and the red value was calculated, resulting in the equation $X = 1.70 - 2.20 r$, with an R^2 value of 0.898. Therefore, to predict the biomass concentration of a culture using its digital image, first, the blue value would be calculated. If it is between 0.2 and 0.9, the biomass concentration would be calculated using the blue value. If, on the other hand, the blue value were less than 0.2, then the red value would be calculated, and if it is greater than 0.3, the red value would be used for biomass calculation. No correlation is recommended if the red value is less than 0.3.

Anabaena variabilis. Figure 3 shows the red, green, and blue values as a function of *A. variabilis* biomass concentration. The color values featured less variation between experimental runs than the *Chlorella* sp. monocultures. This is attributed to the lesser prevalence of flocculation of *A. variabilis*, which led to more homogeneous cultures. Moreover, the red and green values declined more steeply with increasing *A. variabilis* concentration than with increasing *Chlorella* sp. concentration due to the presence of phycocyanin and phycoerythrin in *A. variabilis*, which have absorption peaks at 620 nm and 565 nm, respectively.³⁰

For biomass concentrations less than 0.33 g/L, the red value was more sensitive to biomass concentration than the green or blue values, and was therefore identified as the appropriate parameter for biomass estimation. In this concentration range, the least squares line was defined by the equation $X = 0.434 - 0.512 r$, with an R^2 value of 0.957. At biomass concentrations greater than 0.33 g/L, both the red and the blue values were saturated, whereas the green value was not. Therefore, in the concentration range of 0.33–1.0 g/L, the correlation $X = 1.50 - 1.90 g$ was generated, with an R^2 value of 0.925. The equations and R^2 values of the least squares regression lines are summarized in Table 1.

Accuracy of Biomass Correlations. To evaluate the accuracy of the multispectral imaging method for biomass quantification, independent sets of *Chlorella* sp. and *A. variabilis* cultures with known biomass concentrations were imaged. The biomass concentration was calculated using the correlations shown in Table 1. Figure 4 shows the predicted vs. actual biomass concentration for the two strains. The average error between the actual and predicted biomass concentrations for *Chlorella* sp. and *A. variabilis* was 22 and 14%, respectively. This accuracy is similar to that of the previous imaging method we presented for biomass quantification.²¹ However, the present method expands the measurable biomass range up to 0–1 g/L, which is the range of interest in open pond cultivation systems.

Identification of invasion of a green algae culture by cyanobacteria

Healthy monocultures of *Chlorella* sp. and *A. variabilis* have different red-to-blue ratios, as shown in Figure 5. Therefore, the red-to-blue ratio of a culture can be used to identify invasion of one group by another. For example, invasion of an initially mono culture of *Chlorella* sp. by *A. variabilis* can be detected by sensing a disproportionate decrease in red value compared to the decrease in blue value. To demonstrate this technique, images were acquired of an initially mono culture of *Chlorella* sp. culture at a

biomass concentration of 0.16 g/L as different amounts of *A. variabilis* were added. The red and blue values were calculated for each image. The blue value was also used to calculate the expected red value for a *Chlorella* sp. monoculture. Figure 6 shows the red value of the mixed cultures as a function of the invasion ratio, as well as the red value that would be expected for a monoculture. The figure indicates that

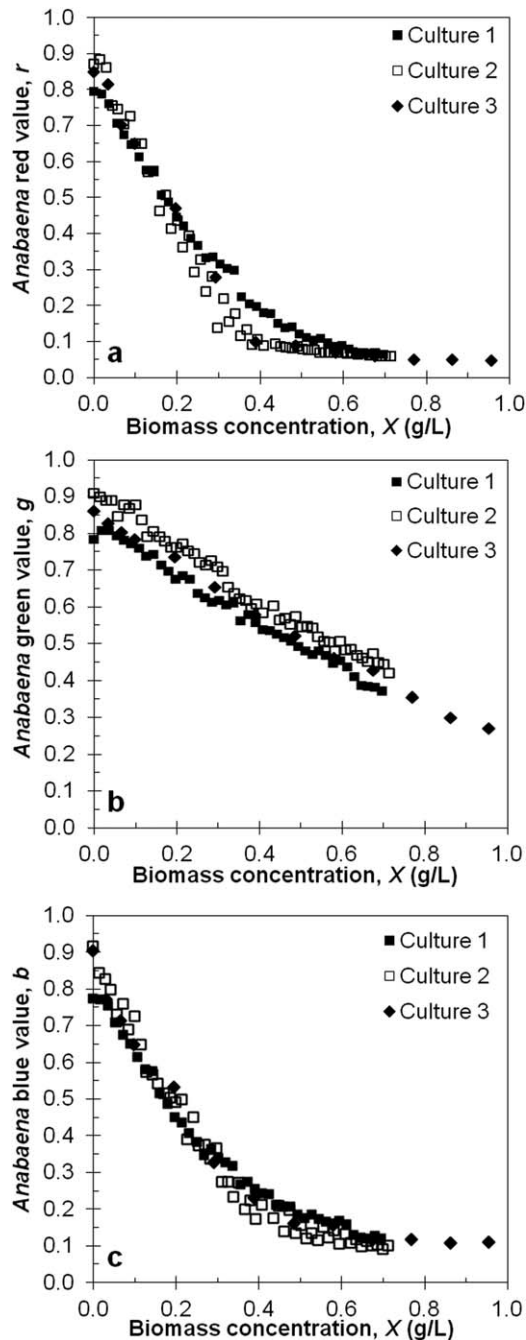


Figure 3. (a) Red, (b) green, and (c) blue values as a function of *A. variabilis* biomass concentration.

contamination by *A. variabilis* was experimentally detected for invasion ratios of 0.08 or greater. Moreover, after interpolating the two data sets, it is expected that invasion ratios as low as 0.04 can be detected.

It should be noted that this invasion detection technique was only tested at a single *Chlorella* sp. concentration. As shown in Figure 5, the lowest invasion ratio that can be detected is a function of the blue value of the monoculture. Smaller invasion ratios can be detected for monocultures with larger differences between the *Chlorella* sp. red value and the *A. variabilis* red value. Therefore, the reference platform presented here is best suited for detecting invasion when the monoculture has a blue value of about 0.25, which corresponds to a biomass concentration of 0.35 g/L. To optimize invasion detection for larger monoculture biomass concentrations, the depth of the submerged plate of the reference platform can be reduced to increase the blue value at a given concentration.

This invasion detection technique can be applied to detect invasion of a monoculture of one organism by another organism so long as the two organisms have different red to green to blue ratios. Moreover, the invasion ratio detection limit can be enhanced by measuring the backscattered light at narrow wavebands corresponding to absorption peaks of pigments distinct to each organism. This can be accomplished using a hyperspectral camera or an RGB camera equipped with narrow bandpass filters. These techniques would be more sensitive to invasion ratio, but also more expensive than using a standalone RGB camera. In large scale applications, invasion detection can be used to inform decisions regarding manipulation of the pH or nutrient concentrations, enabling the species of interest to out-compete the invaders.

Identification of a temperature induced culture crash

RGB images and fluorometric photosynthetic yield parameters were continuously acquired of an *A. variabilis* monoculture at 0.2 g/L as it was heated from 23 to 53°C at a rate of $0.3 \pm 0.03^\circ\text{C}/\text{min}$. Figures 7a and b show the color values and fluorometric parameters, respectively, as a function of culture temperature. At temperatures between 23 and 33°C, all measured parameters were constant. However, between temperatures of 33 and 48°C, the red and blue values increased at an average rate of 0.017 and 0.021°C^{-1} , respectively, meaning that the culture became paler, although this change was imperceptible to the naked eye. In this temperature range, the photosynthetic yield also decreased at an average rate of 0.022°C^{-1} , marking a decline in the health of the culture. Then, at a temperature of 49°C, the maximum fluorescence of the culture abruptly decreased from 53 to 26 a.u., signaling irreversible degradation of Photosystem II. At this same temperature, the red and blue values each abruptly decreased by about 20%. This analysis shows that steadily increasing red and blue values can be used as a “warning sign” of an impending crash caused by thermal stress. Real time image acquisition and analysis can therefore provide information for mitigating such crashes.

Table 1. Correlations Between Biomass Concentration and red (*r*), green (*g*), and blue (*b*) values for *Chlorella* sp. and *A. variabilis*

	<i>Chlorella</i> sp.		<i>A. variabilis</i>	
Color value range	$0.2 < b < 0.9$	$b < 0.2 \text{ \& } r > 0.3$	$0.1 < r < 0.9$	$r < 0.1 \text{ \& } g > 0.3$
Concentration range (g/L)	$0 < X < 0.37$	$0.37 < X < 1.0$	$0 < X < 0.33$	$0.33 < X < 1.0$
Concentration, <i>X</i> (g/L)	$0.477\text{--}0.522 \text{ } b$	$1.70\text{--}2.20 \text{ } r$	$0.434\text{--}0.512 \text{ } r$	$1.50\text{--}1.90 \text{ } g$
<i>R</i> ² value of correlation	0.920	0.898	0.957	0.925

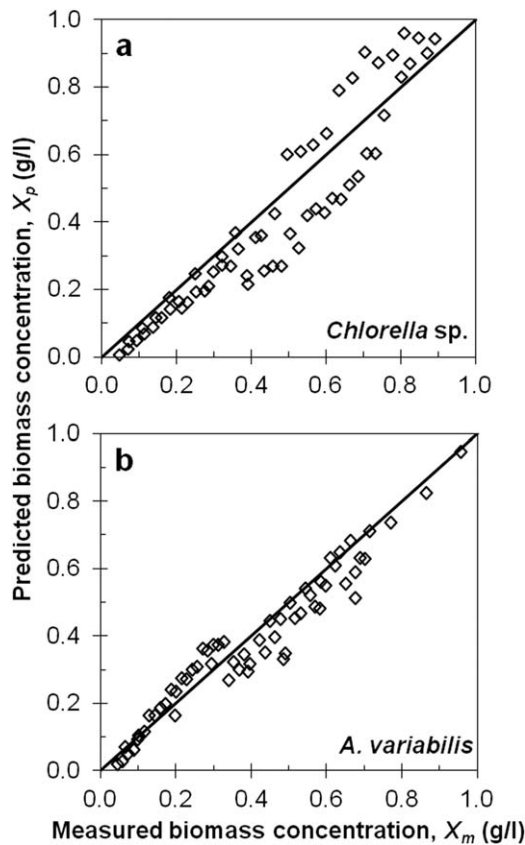


Figure 4. Predicted vs. measured biomass concentration of monocultures of (a) *Chlorella* sp. and (b) *A. variabilis*.

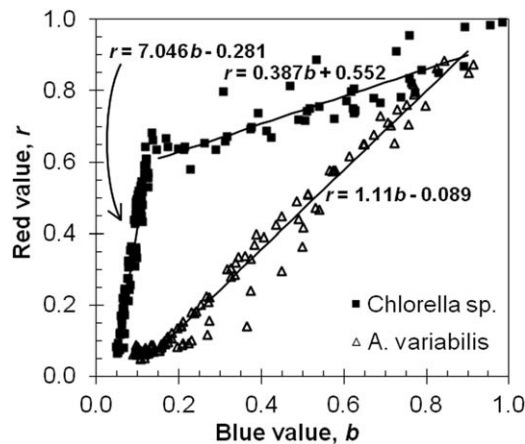


Figure 5. Red value as a function of blue value for monocultures of *Chlorella* sp. and *A. variabilis*.

It should also be noted that the effectiveness of this method for predicting culture crashes is dependent on the biomass concentration. For example, the sensitivities of the red and blue values to *A. variabilis* biomass concentration are much smaller at biomass concentrations greater than 0.4 g/L than they are at concentrations less than 0.4 g/L. It is therefore expected that the red and blue values would also be less sensitive to temperature induced pigmentation changes in this high biomass concentration range. Thus, for applications at operating biomass concentrations greater than about 0.4 g/L, the depth of the submerged plate can be reduced to make the red and blue values more sensitive to pigmentation changes.

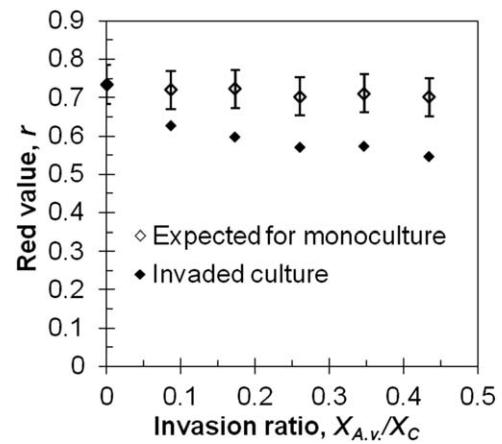


Figure 6. Red values of a *Chlorella* sp. culture with different amounts of *A. variabilis*, as well as the red value that would be expected for a *Chlorella* sp. monoculture.

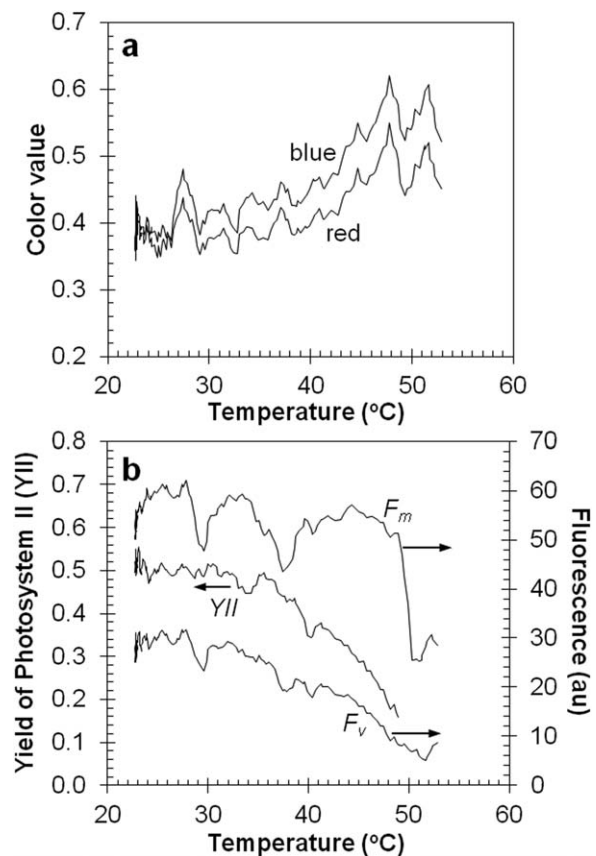


Figure 7. (a) Red and blue values and (b) fluorometric parameters as a function of culture temperature for a monoculture of *A. variabilis*.

The current technique is advantageous over conventional methods in that it enables instantaneous feedback on a wide range of culture parameters, including biomass, invasive species presence, and culture health, over large cultivation areas without the need for culture sampling, and is potentially less expensive. In this way, it can serve as a first level of monitoring and can identify ponds that might need more in depth analysis with other more elaborate and accurate methods that require sampling.

Conclusions

In this article, a multispectral imaging approach was presented for monitoring the backscattered light from algal cultures. The spectral signature of the backscattered light can in turn be used for culture diagnostics such as biomass concentration quantification, invasive species detection, and culture health monitoring. A novel floating reference platform and an RGB camera were used to measure the backscattered light from the cultures. Correlations were generated between the red, green, and blue backscattered intensities and the biomass concentrations of the green alga *Chlorella* sp. and the cyanobacterium *A. variabilis*. These correlations predicted the biomass concentrations of independently prepared cultures with an accuracy of 22 and 14%, respectively. Moreover, a technique was described for using the spectral signature of a green algae culture to detect invasion by cyanobacteria. This technique was successful in detecting cyanobacteria-to-green algae mass ratios as small as 0.08. Finally, a culture crash was simulated by heating an *A. variabilis* culture from 23 to 53°C while simultaneously monitoring the photosynthetic yield and culture color. It was shown that a decrease in the photosynthetic yield coincided with an increase in the red and blue values of the culture image, enabling the red and blue values to be used as a proxy for culture health. These techniques enable real time culture diagnostics, which in turn enable efficient operation of large scale cultivation systems with respect to harvesting, nutrient delivery, and pH and temperature control.

Notation

- b = blue intensity of an image
 \vec{c} = color vector containing the red, green, and blue intensities of an image
 F_m = maximum fluorescence after a saturating pulse
 F_o = baseline fluorescence
 F_v = variable fluorescence
 g = green intensity of an image
 R^2 = coefficient of determination
 r = red intensity of an image
 T = temperature, °C
 X = biomass concentration, g/L
 $Y(II)$ = quantum yield of photosystem II

Subscripts

- A_v = refers to *Anabaena variabilis*
 C = refers to *Chlorella* sp.
 g = refers to green region
 p = refers to pixel
 w = refers to white region

Abbreviations

- PAM = pulse-amplitude modulated
 PAR = photosynthetically active region
 POC = particulate organic carbon

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