Multispectral Image Analysis for Algal Biomass Quantification

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This article reports a novel multispectral image processing technique for rapid, noninvasive quantification of biomass concentration in attached and suspended algae cultures. Monitoring the biomass concentration is critical for efficient production of biofuel feedstocks, food supplements, and bioactive chemicals. Particularly, noninvasive and rapid detection techniques can significantly aid in providing delay-free process control feedback in large-scale cultivation platforms. In this technique, three-band spectral images of Anabaena variabilis cultures were acquired and separated into their red, green, and blue components. A correlation between the magnitude of the green component and the areal biomass concentration was generated. The correlation predicted the biomass concentrations of independently prepared attached and suspended cultures with errors of 7 and 15%, respectively, and the effect of varying lighting conditions and background color were investigated. This method can provide necessary feedback for dilution and harvesting strategies to maximize photosynthetic conversion efficiency in large-scale operation. © 2013 American Institute of Chemical Engineers *Biotechnol. Prog.*, 29:808–816, 2013
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Introduction

Algae biomass cultivation has a multitude of applications, including wastewater remediation, carbon dioxide mitigation, and biofuel production.[1–3] Algae can be cultivated as suspended cultures in a liquid medium or attached cultures to a solid or porous substrate, with each regime having distinct advantages and disadvantages.[4,5] Photobioreactors and open ponds, which employ suspended cultures, enable fast gas and nutrient transfer rates to and from the cells, which enhances productivity but requires large water volumes and energy inputs for operation.[6,7] On the other hand, photobioreactors employing attached cultures offer high microorganism concentrations, thus diminishing the water and energy input requirement but often suffer from lower biomass productivities due to mass transfer limitations.[4] Many algal strains, including the cyanobacteria *Anabaena variabilis*, have been shown to grow as both attached and suspended cultures.[8,9]

Efficient operation of algae ponds and photobioreactor fields requires an accurate, real-time biomass quantification method that can operate under different lighting conditions and over large areas. The local photosynthetic rate in these cultivation systems is strongly dependent on the local irradiance.[10,11] The local irradiance in absorbing and scattering media such as algae cultures is most accurately described by the radiative transport equation (RTE) which correctly accounts for the scattering phenomena. The solution of RTE yields an exponential decay in irradiance with increasing optical depth from the irradiated surface.[12,13] Noting that local optical depth is directly proportional to the microorganism concentration,[13] real-time quantification of microorganism concentration enables implementation of mixing, dilution, and harvesting strategies to maintain the photobioreactor optical thickness at a level that maximizes the total photosynthetic productivity of the cultivation system.

This article presents a rapid, noninvasive, and inexpensive multispectral imaging technique for measuring the biomass concentration of algae cultures. The technique uses a conventional RGB camera and a computer code for multispectral image analysis to quantify the areal biomass concentration of both attached and suspended cultivation systems. The large view field of image acquisition enables the technique to be applied for monitoring large-scale systems and algae cultivation farms. Moreover, implementation of time-lapse quantification of biomass concentration enables real-time productivity monitoring.

Current State of Knowledge

Biomass quantification in suspended cultures

The biomass concentration of suspended cultures is conventionally measured either by direct biomass weighing of a culture sample, or by measurement of a proxy for biomass, typically optical density or chlorophyll concentration.[14] Dry biomass weighing entails weighing an empty, dry filter, filtering a liquid sample through the filter, drying the sample, and reweighing it.[15] This method is very simple, but it requires an oven, filter paper, a filtering apparatus, and a scale. Further, the dry biomass concentration of the sample is dependent on the sampling location. Moreover, nonalgal microorganisms and salts can be retained in the filtering

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process and counted as dry biomass, resulting in overestimation of the algal biomass concentration.[15] Finally, the drying time is usually several hours, thus precluding the possibility of real-time biomass quantification.

Direct measurement of monochromatic optical density is often used as a proxy for biomass concentration.[14] Optical density of a culture is measured at a specific wavelength in a spectrophotometer and correlated to biomass concentration using published calibration curves. However, the accuracy of said correlations is dependent on measurement specifications such as the spectral bandwidth and acceptance angle of the measuring instrument as well as the spectral distribution of the light source.[16] Furthermore, the optical density of the sample can be dependent on sampling location.

Chlorophyll extraction entails centrifuging a sample, resuspending the concentrated sample in a solvent (usually ethanol or methanol), and measuring the optical density of the resulting chlorophyll suspension at specific wavelengths in a spectrophotometer.[14] The chlorophyll concentration is calculated from the optical density using published correlations.[17] This process can be performed in less than an hour, but it requires solvents, a centrifuge, and a spectrophotometer. Moreover, the accuracy of the results depends on the similarity between the spectral content of the light source and the spectral bandwidth of the measuring instrument and the instrument used in obtaining the published correlations.[16]

More recently, Jung and Lee[18] reported a method for using image analysis to measure the biomass concentration of a vertical tubular bioreactor. In their method, a red-green-blue image taken from the top of the reactor was converted to a grayscale image. The average gray value of the grayscale image was then correlated to the biomass concentration. However, in their study, the vertical tubular photobioreactor was illuminated from the sides and imaged from the top. Therefore, the average gray value was dependent on tube diameter, as a culture in a larger diameter tube will appear darker than the same culture in a smaller diameter tube. Additionally, the correlation is also dependent on the spectral content of the light source, as an algae culture illuminated with green light will have a higher average gray value than one illuminated by red or blue light due to selective absorption by the photosynthetic pigments.[19] Moreover, on a large scale, illumination from the top of a culture is logistically more feasible than uniform illumination from all sides.

Biomass quantification of biofilms

Norrman et al.[20] presented an electromechanical method for measuring the thickness of a biofilm growing on a metal surface. In this method, the authors incrementally lowered a needle cathode toward the biofilm surface, and the locations of the air-biofilm interface and biofilm-substrate interface were determined by abrupt changes in the current through the cathode. Although this method is reliable and accurate for laboratory applications, its practicality at photobioreactor scales is a concern.

Additionally, several noninvasive optical methods for measurement of nonphotosynthetic biofilm thickness and biomass concentration have been reported.[21–23] Bakke and Olson[21] presented a method in which a light microscope was focused first on the biofilm-substratum interface and then on the biofilm-liquid interface. The biofilm thickness was recovered from the distance between the two focal planes. Moreover, the same group presented a monochromatic imaging method for measuring biofilms' total organic carbon content per unit area.[23] They grew a biofilm of the nonphotosynthetic bacteria *Pseudomonas aeruginosa* in a rectangular duct bioreactor. They then found a linear correlation between the biofilm's total organic carbon per unit area and its optical density at 420 nm. The aforementioned methods can be used to quickly measure biofilm thickness, but they require a transparent substratum and either a microscope or a monochromatic, collimated light source, and photometer. Furthermore, photosynthetic biofilms challenge the applicability of these methods because absorption by pigments impedes light transmission through the biofilm.

Kazemipour et al.[24] measured the spectral reflectance and transmittance of microphytobenthic biofilms in an effort to determine their biomass concentration using remote hyperspectral imaging. The inherent reflectance and transmittance of simulated biofilms of the diatom *Entomoneis paludosa* were calculated by measuring the apparent reflectances of the biofilms on two backgrounds with different known reflectances. The absorption coefficient at 673 nm, corresponding to absorption by chlorophyll a, was calculated from these parameters and correlated with biomass concentration. The biomass concentration of an independently prepared set of biofilms was predicted with a coefficient of determination R^2 value of 0.93 by measuring the biofilms' apparent reflectance at 673 nm. This method enables noninvasive biomass quantification and is independent of the intensity and spectrum of the light used to illuminate the biofilm. However, the method requires the use of a hyperspectral camera, which can be cost-prohibitive in many applications.

As demonstrated by Jung and Lee, it is possible to recover the biomass concentration of suspended cultures using an inexpensive camera. However, their correlation was specific to the lighting conditions and the geometry of the photobioreactor used, limiting the applicability of this method and the reported correlation in other systems. Thus, imaging methods that can quantify biomass concentration and are independent of the geometry of the photobioreactor and the light source used are needed. Moreover, individually analyzing the red, green, and blue intensities of an algae culture image and comparing them to those of a white reference can eliminate the dependence of the image analysis technique on the spectral content of the light source. This article reports, for the first time, a rapid, noninvasive, inexpensive method for determining the biomass concentration of both suspended and attached cultures using wideband multispectral imaging of reflected and backscattered light.

Materials and Methods

Stock culture cultivation

The cyanobacteria *A. variabilis* (ATCC 29413-U) was used as an exemplary microorganism due to its widespread use in experimental studies on photobiological CO_2 mitigation and biohydrogen production.[8,9,25] *A. variabilis* is a cyanobacteria composed of cells of approximately 5 μm in diameter forming filaments more than 100-μm long.[19] Its cultivation as both suspended and attached cultures have been shown.[8,9] Also, the pigmentation and the optical properties of *A. variabilis* have been reported.[19,26] Optically, the organisms have absorption peaks at 440 and 680 nm,

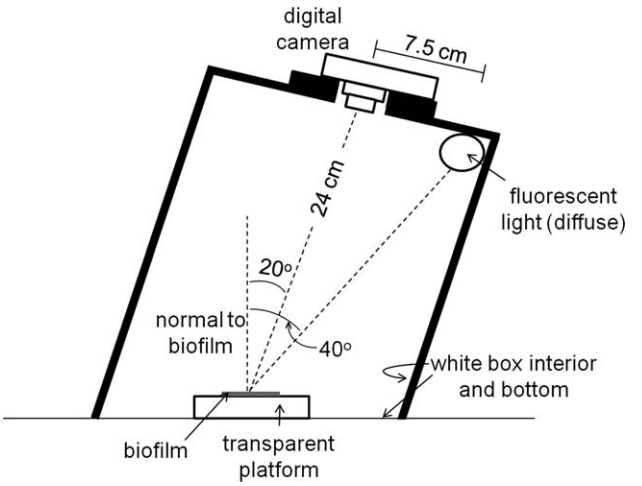

Figure 1. Cross-section schematic of the photobox.

corresponding to chlorophyll a, as well as an absorption peak at 620 nm, corresponding to phycocyanin, a light-harvesting phycobiliprotein.[19,26] In lesser amounts, *A. variabilis* also contains carotenoids, with broad absorption between 400 and 500 nm, and the phycobiliproteins, phycoerythrin and allophycocyanin, with absorption peaks at 565 and 650 nm, respectively.[26] The stock suspended culture for the experiments was cultivated in BG11 nutrient medium,[14] sparged with air containing 2% by volume carbon dioxide, and continuously illuminated with 16 ± 2 W/m^2 irradiation (74 ± 8 μE/m^2/s) in the photosynthetically active region (PAR) using cool white fluorescent bulbs (Philips, F32T8).

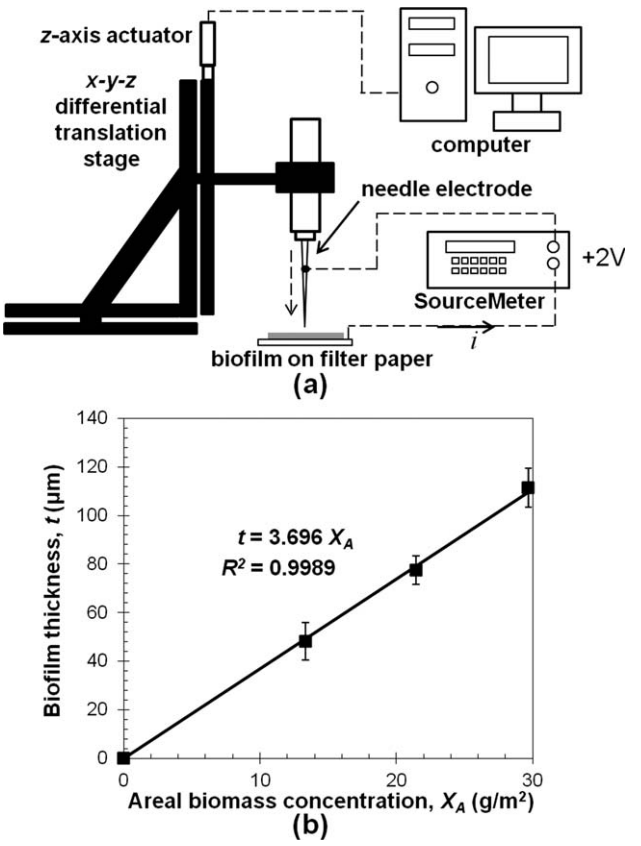

Figure 2. (a) Schematic of the experimental setup used for measuring biofilm thickness and (b) biofilm thickness vs. areal biomass concentration.

Biofilm preparation and imaging

Two suspended stock cultures, one 5-days old and the other 7-days old, were used to generate the biofilms to evaluate the effect of culture age on image analysis results. For each culture, biofilms of varying thickness were simulated by filtering a known volume of microorganism suspension from the stock culture onto glass fiber filter paper with an average pore diameter of 0.7 μm (Whatman, GF/F) using a vacuum filtration apparatus (Kimax, 27070). The resulting biofilm had a diameter of 3.8 cm. Then, the biofilm was placed into a custom-made illuminated box (photobox) to ensure that each image acquired was subject to the same background and lighting conditions (Figure 1). Inside the photobox, the biofilm was illuminated with diffuse light provided by a fluorescent bulb (Underwriters Laboratories, Portable Luminaire) at an irradiance of 4.5 W/m^2 (21 μE/m^2/s). A digital camera with 8-megapixel resolution (Logitech, Pro 9000) was then placed into the camera port of the photobox. The automatic exposure and white balance features were disabled to avoid automatic increases in image brightness as the culture became darker on addition of microorganisms. The exposure and white balance were set to their minimum values and the gain was set to its maximum value. These settings were selected because they produced the greatest color contrast possible in the image.

After each image acquisition, an incremental volume of suspended stock culture was added to the biofilm by vacuum filtration. This process was repeated until there was no noticeable change in biofilm appearance. It was ensured that each volume of suspended stock culture filtered onto the paper had the same microorganism concentration by stirring the stock culture prior to each filtration. Given the short duration of the experiments (less than 5 min per sample) and the low light levels used (less than 21 μE/m^2/s), no appreciable variations in either the biomass concentration or the optical properties of the cells were expected.

Biofilm thickness and areal biomass concentration

To measure biofilm thickness t, a modified version of the method presented by Norrman et al.[20] was used. In this method, a needle electrode was maintained at 2 V bias with respect to the biofilm-supporting filter paper using a SourceMeter (Keithley 2400; Figure 2a). With the needle electrode in air, the current through the circuit was at its noise level of approximately 20 pA. The needle electrode was lowered toward the biofilm surface at a rate of 1 μm/s using a three axis differential translation stage (Thorlabs, PT3A) and a computer-controlled actuator (Thorlabs, ZST25B and TST001). The needle was lowered until the current through the circuit abruptly increased by four orders of magnitude to approximately 0.1 μA. This signaled the completion of the circuit, and the vertical needle position corresponded to the top of the biofilm. This process was repeated for the nonbiofilm-supporting region of filter paper. In this case, the vertical needle position at which the circuit was completed marked the bottom of the biofilm. For a given biofilm, this process was repeated at five biofilm locations to enable calculation of the spatial variance of the biofilm thickness.

Finally, the areal biomass concentration X_A of the biofilms was calculated as the ratio of the dry algal biomass to the biofilm area. For this, the volumetric biomass concentration

of the stock culture used in the experiments was determined according to the standard methods reported.[15] This was done simultaneously while preparing the biofilms to eliminate the effects of growth. The dry biomass of each biofilm was then calculated by multiplying the volumetric biomass concentration of the suspended stock culture with the volume of culture used to make the biofilm.

Suspended culture preparation and imaging

The chamber for holding suspended cultures was a custom built acrylic box that measured 10.0 × 6.4 × 8.1 cm^3 in length, width, and height, respectively. The top of the box was open to create a top-irradiated illumination scheme typical of scaled-up open raceway ponds, and the sides were covered with white paper to impose symmetry boundary conditions. Cultures of different biomass concentrations having a total volume of 400 mL were placed in the acrylic box and imaged. The concentration of the culture in the acrylic box was measured by filtering a known volume onto filter paper, drying, and weighing.

Image analysis

A custom computer code was developed to analyze the images of the algal cultures. 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.[27] 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 only the algal culture (green region) was identified. The raw color vector of the green region $\vec{c_o}$ equal to $[r_o, g_o, b_o]$ was then calculated as the average of the red, green, and blue intensities of all the pixels in the region. Then, a region of the image that contained a white reference background was identified. The biofilm-supporting filter paper and the white sides covering the acrylic box were used as the white reference regions for the images of the attached and suspended cultures, respectively. The color vector of the white reference 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. The elements of the normalized color vector $\vec{c}$ used in the analysis were calculated by dividing each raw color intensity by the intensity of that color in the white region to account for differences in the intensity and spectral content of the light source:

$$\vec{c}(i) = \vec{c_o}(i)/\vec{c_w}(i) \qquad \text{for } i = 1, 2, 3 \tag{1}$$

Results and Discussion

Relationship between biofilm thickness and areal biomass concentration

Figure 2b shows the relationship between the areal biomass concentration and the biofilm thickness. A linear relationship was recovered using the electromechanical method. The thickness t is given in terms of areal concentration X_A as, $t = 3.696\ X_A$, where t is in μm and X_A is in g/m^2. The coefficient of determination R^2 for this fit was 0.9989. Using these results, it was established that the volumetric microorganism concentration in the biofilm was 271 kg dry biomass per cubic meter (kg DW/m^3).

Attenuation of red, green, and blue light in the algal cultures

Figure 3 shows the normalized red, green, and blue intensities (r, g, and b) of attached and suspended cultures as a function of areal biomass concentration. The magnitude of each color intensity of a given image is a result of the combined effects of reflection from the culture surface and backscattering from within the culture. The intensity of the back-scattered light is governed by the RTE, which takes into account absorption and anisotropic scattering by the microorganisms and the medium.[12,28] Solving the RTE for the backscattered intensity from dense cultures poses a challenge as scattering phenomena gets into dependent regime where the radiative properties of the culture can no longer be described as linear functions of microorganism concentration.[12] Therefore, this analysis seeks an empirical correlation between areal biomass concentration and color intensity, taking into account reflected and back-scattered light from the culture. The equation form for the empirical model has been formulated by modeling light transfer through the algae culture with ray tracing analysis[24] and is given by,

$$\vec{c}(i) = \vec{\alpha}(i) + \vec{\beta}(i) e^{-2X_A E_{p,ext}(i)} \tag{2}$$

where the vector $\vec{\alpha}$ represents the reflected intensity from the surface of the culture, and is assumed to be independent of biomass concentration based on the results presented by Kazemipour et al.[24] Moreover, the vector $\vec{\beta}$ represents the reflectance of the bottom surface of the algae culture. The vector $\vec{E}_{p,ex}$ contains the pseudo-mass extinction cross sections for red ($i = 1$), green ($i = 2$), and blue ($i = 3$) light, which control the attenuation of irradiance of each color along the ray path.

The experimental data shown in Figure 3 were fitted with Eq. 2 using the linear least-squares method.[29] Table 1 summarizes the values of $\vec{\alpha}$, $\vec{\beta}$, and $\vec{E}_{p,ex}$, as well as the R^2 value for each fit. All R^2 values obtained were greater than 0.97. The wideband pseudo-extinction cross sections of the red and blue bands were approximately equal to each other and greater than that for the green band by a factor of about 3.5. The more gradual attenuation of backscattered green light compared to red and blue light is a result of the relatively low absorptivity of *A. variabilis* at wavelengths between 490 and 590 nm, which is in turn a result of the organisms' lack of photosynthetic pigments that absorb light in that wavelength range.[19,26] Moreover, the pseudo-extinction cross sections for the suspended cultures were greater than those for the attached ones by an average of 35%. This can be attributed to (i) light absorption by the liquid medium of the suspended culture and (ii) to dependent scattering in the biofilm, decreasing the effective extinction compared to that in an independently scattering medium.[28]

Furthermore, in both cultures, the color intensities at areal biomass concentrations greater than 10 g/m^2 were greater than their respective intensities at areal biomass concentrations between 8 and 10 g/m^2 by approximately 0.02. This trend is especially clear for the red and blue intensities of the suspended cultures. This trend can be attributed to the increase in backscattering that resulted from multiple scattering by organisms in a thin layer below the illuminated surface at higher concentrations. Finally, the components of the vector $\vec{\alpha}$ were greater for the suspended cultures than for the attached ones by a factor of about 5, indicative of the greater surface reflectivity of water compared to a microorganism film layer.[24,28]

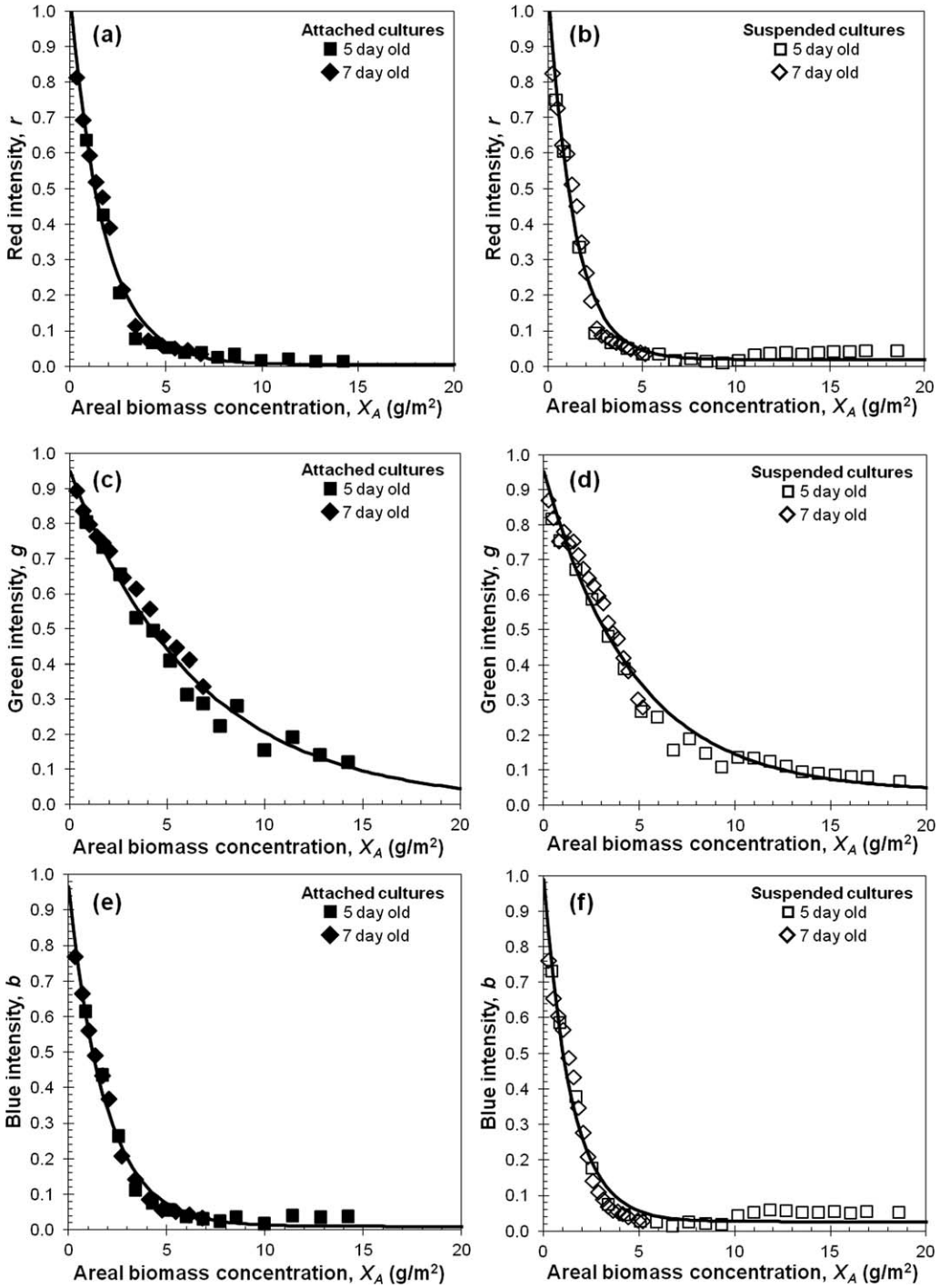

Figure 3. Normalized color intensity as a function of areal biomass concentration for attached and suspended cultures at two culture ages for (a,b) red, (c,d) green, and (e,f) blue.

Table 1. The curve fit parameters α and β, wideband pseudo-mass extinction cross sections $E_{p,ext}$, and coefficient of determination R^2 for fitting Eq. 2 to the experimental data

	Red Band 560–700 nm		Green Band 490–590 nm		Blue Band 410–500 nm	
	Attached	Suspended	Attached	Suspended	Attached	Suspended
α	0.005	0.019	0.002	0.038	0.009	0.026
β	1.03	1.05	0.919	0.915	0.956	0.963
$E_{p,ext}$ (m^2/g)	0.281	0.365	0.077	0.107	0.269	0.346
R^2	0.984	0.974	0.981	0.977	0.989	0.972

Table 2. Areal biomass concentration ($X_{A,f}$), biofilm thickness (z_a), and suspended culture physical depth (z_s) at which the wideband red irradiance is attenuated to 10, 1, and 0.1% of its value at the light-facing side of the culture, assuming attached and suspended volumetric microorganism concentrations of 271 kg/m^3 and 500 g/m^3, respectively

	Attached Cultures		Suspended Cultures	
f	$X_{A,f}$ (g/m^2)	z_a (m)	$X_{A,f}$ (g/m^2)	z_s (m)
10%	8.2	3.0×10^{-5}	6.3	0.013
1%	16.4	6.1×10^{-5}	12.6	0.025
0.1%	24.6	9.1×10^{-5}	18.9	0.038

The rate at which light energy is converted to chemical energy in photosynthetic systems is a function of the wavelength, known as the photosynthetic action spectrum, as well as the local irradiance.[30] Thus, knowledge of spectral light attenuation within a culture can provide valuable information about the overall productivity of that system. Particularly, the local productivity in *A. variabilis* cultures is highly dependent on the availability of red light as its photosynthetic action spectrum indicates one predominant peak at a center wavelength of 633 nm and a half width at half maximum of 37 nm.[30,31] Therefore, using the wideband red pseudo-extinction cross section obtained in this study, we can illustrate the areal biomass concentration, $X_{A,f}$, for *A. variabilis* at which the local red irradiance drops to 10, 1, and 0.1% of its value incident on the culture as,

$$X_{A,f} = -\ln(f)/E_{\mathrm{p,ext}}(\mathrm{red}) \tag{3}$$

where f represents the attenuation fraction. In this analysis, it is assumed that the culture density is such that all red irradiance is absorbed before reaching the back surface. Table 2 presents the areal biomass concentrations at which the wideband red irradiance is attenuated to 10, 1, and 0.1% of its incident value within attached and suspended cultures of *A. variabilis*. The table also shows the associated biofilm thicknesses and suspended photobioreactor depths that correspond to these concentrations, assuming attached and suspended volumetric microorganism concentrations of 271 kg/m^3 and 500 g/m^3, respectively.[32]

Correlation between normalized green intensity and areal biomass concentration

The normalized green intensity displayed more gradual attenuation with increasing biomass concentration than the red and blue intensities. Therefore, the normalized green intensity was identified as the appropriate value to correlate to areal biomass concentration because such a correlation would be accurate within a larger range of concentrations. For attached cultures, rearranging Eq. 2 and using the coefficient values from Table 1 yields,

$$X_A = -6.49 ln\left(\frac{g - 0.0016}{0.947}\right) \tag{4}$$

Equation 4 is recommended for areal biomass concentrations between 0.34 and 14 g/m^2, which was the range of areal biomass concentrations examined in this study. Similarly, for suspended cultures:

$$X_A = -4.69 ln\left(\frac{g - 0.0375}{0.915}\right) \tag{5}$$

Equation 5 is recommended for areal biomass concentrations between 0.25 and 21 g/m^2.

It is worth noting that the coefficients in Eqs. 4 and 5 are dependent on the cellular pigment concentrations. It is well-known that cells can up- or down-regulate their pigment contents depending on cultivation conditions.[33] However, it is possible to reestablish the pigment-biomass correlation as necessary to account for these effects.

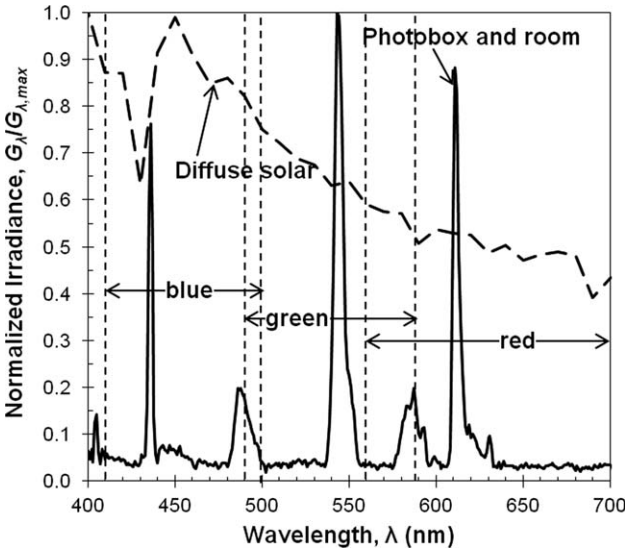

Figure 4. Normalized spectral intensities of the three light sources used for image acquisition, along with the wavelength bands of the color filters of the RGB camera.[27,34]

Validity of the correlation under different lighting conditions and backgrounds

To take full advantage of this biomass quantification method, it must be insensitive to the magnitude, spectral content, and angle of incidence of the irradiance onto the culture, as well as to the spectral reflectance of the background surrounding the culture. Therefore, the accuracies of Eqs. 4 and 5 were measured on independently prepared sets of attached and suspended culture images acquired using combinations of three different light sources and two different backgrounds. The three light sources used were the fluorescent lamp of the photobox, fluorescent room lighting, and shaded diffuse solar illumination. The hemispherical PAR (from 400 to 700 nm) for the photobox, room, and sunlight were measured with a quantum sensor (Li-Cor, LH-100) to be 4.5 W/m^2 (21 μE/m^2/s), 1.3 W/m^2 (6.0 μE/m^2/s), and 1.6 W/m^2 (7.4 μE/m^2/s), respectively. The normalized spectral intensities of the three light sources are shown in Figure 4. The spectra of the two fluorescent bulbs were measured using a monochromator (Newport, Cornerstone 260) with 3.7-nm spectral resolution, while the diffuse solar spectrum was reported by Gueymard et al.[34] The two backgrounds were white paper (OfficeMax, Copy Paper) and black epoxy resin lab bench surface (VWR). Black and white materials were selected as backgrounds because the total reflectance in the visible range of any other color material will be between those of these two extremes.

Figure 5a shows digital images acquired of the benthic cultures at an areal biomass concentration of 7.8 g/m^2. The color and brightness of each biofilm appears different to the naked eye due to the variations in the magnitude and spectral content of the incident irradiance. Figure 5b indicates that the benthic culture areal biomass concentration was predicted well by Eq. 4 under all six lighting conditions. Moreover, Table 3 shows the root mean square deviation (RMSD)

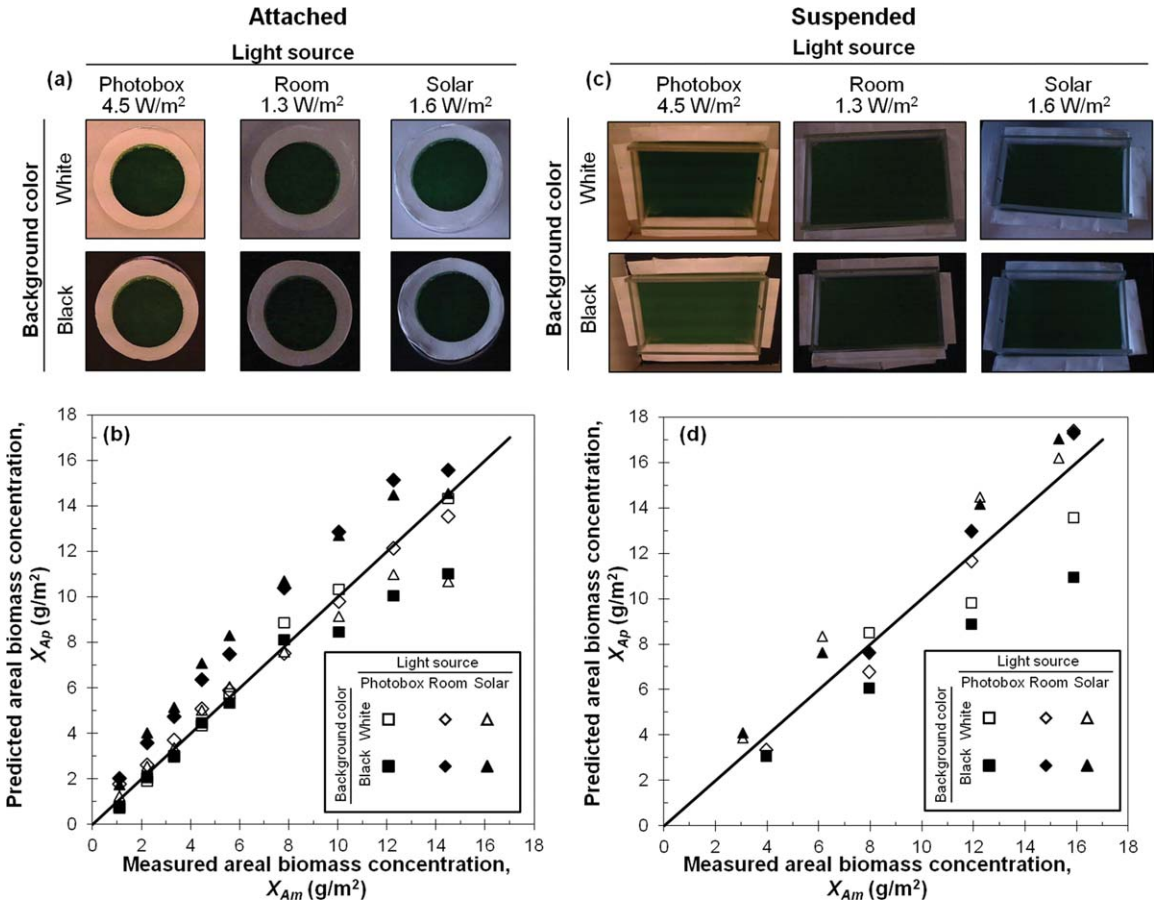

Figure 5. Top: digital images of an (a) attached culture at 7.8 g/m^2 and (c) suspended culture at 6.1 g/m^2 under the six lighting and background combinations. Bottom: predicted areal biomass concentration plotted against measured areal biomass concentration for (b) attached and (d) suspended cultures under the six background and lighting combinations.

Table 3. Root mean square deviation (RMSD) between the areal biomass concentration predicted by Eq. 4 and the actual areal biomass concentration for the six lighting and background scenarios for the attached cultures

Light Source	Background	RMSD for X_A Less than 10 g/m^2 (g/m^2)	RMSD for X_A greater than 10 g/m^2 (g/m^2)
Photobox	White	0.62	0.80
Photobox	Black	0.30	2.10
Room	White	0.72	0.34
Room	Black	2.10	3.39
Solar	White	0.58	1.98
Solar	Black	2.57	2.78

between the areal biomass density predicted by Eq. 4 and the actual biomass density for each of the six conditions. For the case of photobox lighting with white background, which served as the validation data set, the RMSD was 0.67 g/m^2, which corresponds to an average error of 6.5%. The RMSD was highest for solar illumination with black background and room illumination with black background. The average percent error incurred in using Eq. 4 to predict areal biomass density across all six lighting and background scenarios was 25%. Similarly, Figure 5c shows the digital images acquired of the planktonic cultures at an areal biomass concentration of 6.1 g/m^2. Figure 5d shows the areal biomass concentration as a function of normalized green intensity for these cultures,

Table 4. Root mean square deviation (RMSD) between the areal biomass concentration predicted by Eq. 5 and the actual areal biomass concentration for the six lighting and background scenarios for the suspended cultures

Light Source	Background	RMSD for X_A less than 10 g/m^2 (g/m^2)	RMSD for X_A greater than 10 g/m^2 (g/m^2)
Photobox	White	0.73	2.21
Photobox	Black	1.47	4.09
Room	White	0.92	1.09
Room	Black	0.32	1.26
Solar	White	2.41	3.21
Solar	Black	2.00	2.91

as well as the areal biomass concentration predicted by Eq. 5. Moreover, Table 4 shows the RMSD between the areal biomass density predicted by Eq. 5 and the actual biomass density for each of the six conditions. For the case of photobox lighting with white background, which serves as the validation data set, the RMSD was 1.64 g/m^2, which corresponds to an average error of 15%. The RMSD was highest for solar illumination with both white and black backgrounds. The average percent error incurred in using Eq. 5 to predict areal biomass density across all lighting and background combinations was 21%.

Although the traditional methods can provide more accurate results, they can take long analysis time and/or be highly sampling location-specific as explained previously. Thus, the

temporal and spatial variations in biomass concentration in cultivation systems can blur the accuracy advantage of these more traditional methods with respect to the method presented here. Moreover, in this study, the lighting and background conditions have been identified that minimize the prediction errors. Thus, in practical implementation of the presented method, care can be taken to stay in these higher accuracy regimes.

Conclusions

This article reported a rapid, noninvasive method of biomass quantification in suspended and attached algae cultures using only a simple RGB camera and custom image analysis software. By parsing images of *A. variabilis* cultures into their red, green, and blue components and correlating the green intensity with biomass concentration, the biomass concentrations of independently prepared cultures imaged under a variety of lighting and background conditions were predicted with an average error of 23%. Future work will focus on evaluating the performance of this technique in outdoor photobioreactors, as well as performing time lapse image acquisition for productivity monitoring.

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Notation

b = blue intensity of an image
$\vec{c}$ = color vector containing the elements r, g, and b
$\vec{E}_{p,ex}$ = vector containing wideband pseudo-mass extinction cross sections, m^2/g
f = fraction of local irradiance to incident irradiance
G = irradiance, W/m^2
g = green intensity of an image
r = red intensity of an image
R^2 = coefficient of determination
t = biofilm thickness, μm
X_A = areal biomass concentration, grams dry weight (g DW)/m^2
z = culture depth, m

Greek symbols

α = coefficient for curve fit in Eq. 2
β = coefficients for curve fit in Eq. 2
λ = wavelength, nm

Subscripts

o = refers to raw color vector
p = refers to pixel
w = refers to white region

Abbreviations

PAR = photosynthetically active radiation
RMSD = root mean square deviation

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