Supplementary materials: Underwater multispectral laser serial imager for spectral discrimination of algal and coral substrates

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The following additional Methods (Additional methods M1) describe in more detail the configuration and operation principles of the multispectral laser serial imager components used in this study. These descriptions are followed by an example calculation of the photon budget (Additional methods M2) that may be used in determining the Yield of Fluorescence in different imaged substrates when laser power can be closely monitored, as well as Tables S1 through S4 containing values which support these calculations and those used for the estimation of reflectance and practical fluorescence efficiency. Finally, Figure S1 shows sample images taken via 405 nm laser excitation but that did not provide adequate results for normalization: elastic (reflectance) and inelastic (fluorescence) images are shown with apparent fluorescent response in macroalgae.

Additional methods M1

Multispectral laser source

Assembling the emitter requires aligning and collimating 3 continuous wave (CW) laser diode beams (450 nm, 490 nm, 520 nm) into a galvanometric scanning instrument. The choice of laser sources is based on the absorption and light emission curves of macroalgae and coral (i.e., in the visible spectrum), as coincidentally as well as their transmission in water. The lasers were also chosen for the beam quality, allowing focusing for high resolution imaging in underwater conditions (i.e., 0.5 mm - 1 mm - 2 mm spot size), adjusted to a distance of 2.3m. Laser diode specifics are specified in Appendix A Table A1. During assembly, laser diodes were pressed into 12.5 mm diameter copper housings, and each fitted with a constant current driver. This provided a certain protection from electrostatic discharge as well as help maintain stable power output, as laser diode can pull more current by a gradual inner temperature increase through prolonged operation. These copper housings were also fitted into machined aluminum cylinders measuring 2.54 cm diameter, as a way of providing additional heatsinking and to fit into standard 2.54 cm diameter kinematic mounts. It was originally planned to add a 505nm laser diode to the emitter array for an additional excitation wavelength but was omitted due to time constraints.

Laser canning assembly

The galvanometric mirror system consists of a 3-axis system (i.e., FARO – Nutfield Technology 3XB 3-axis Scan Head + Surfboard-1 Scan Controller). Of the three (X, Y and Z) axes, the Z axis, is the depth or the distance between the Y-axis mirror and the target. A linear lens system can dynamically correct for the curvature of the focal plane during scanning by expanding/reducing beam diameter appropriately at the target range. This effect field curvature effect can be noticeable when scanning at larger scan areas (e.g. 15 cm x 15 cm vs 90 cm x 90 cm). The correction also provides focus enhancement, and spot size on the target, and resolution. However, this feature was not used since the already small laser diode beam diameters did not allow its use without the addition of beam expanders. Nevertheless, the size of the focal point at a distance of 2.3 m, distance between the scanning device and the target, was reasonable for the purposes of the experiment. The 3-axis scanner was controlled via Waverunner software version 3.8.0.0002, in which a scan pattern was programmed while controlling for distance to target, scan speed, line spacing, scan pattern (i.e., left to right, then back to left start of line). Scan area was approximately 100 cm x 100 cm. A start of line signal is sent to the ADC to create the image file by synchronizing scanning and data acquisition to the proper data storage format (National Instruments – TDMS format).

Optical sensor assembly

A Hamamatsu PMT model R9880U-20 detector was inserted into a 3D printed polycarbonate cylinder housing. Appropriate position of the PMT within the housing was determined by optimizing the image footprint over the sensor to reduce chances of overloading the sensor. This housing was affixed to the opening of an electronically controlled and optically sealed 6 position rotary filter wheel. Filters used were no filter, 450nm, 488nm, 520nm, 580nm and 685 nm (see Table S1). The PMT output was wired to a 16-bit digital to analog converter acquisition system.

Data acquisition

Data was acquired using a user-built laser imaging Graphical User Interface (GUI) created in Labview 18.0, where it was possible to program for adjusting sample rate, X and Y resolution, PMT voltage, PMT measured current. For the imaging purposes of this study, a PMT voltage of 650 volts was set as a way of maximizing the fluorescence response in the algal targets while remaining below the upper operational generated current threshold of the PMT. The GUI provided control of the Waverunner scanner software interface via a start of scan signal. The analog to digital conversion of the received signal was done using a National Instruments USB-6366 multifunction ADC, capable of digitizing at 16-Bit resolution and analog input sample rate up to 2 MS/s (see table 1).

Additional methods M2

Photon budget calculations

Irradiance on the pixel - photon model for CW line scan

An important feature to evaluate in the multispectral laser serial imager is the number of incident photons per pixel, otherwise defined as irradiance on the pixel. For such, the description of a photon model is useful to correctly estimate wavelength-dependent elastic backscatter (i.e., reflection) and inelastic (i.e., fluorescence) photons through the imaging medium, as well as determining pixel dwell-time. Additionally, real-life image pixel dimensions must be properly estimated since they may vary as scanned area dimensions can change as distance to target varies (e.g., moving imaging platform over unequal terrain).

Pixel dwell time

Since this multispectral imager records spectral response by creating a 1000 x 1000 pixel image, various methods can be used to obtain energy per time unit or in this case, pixel. As there was no additional sensor monitoring laser output power during the actual imaging scans, laser power was recorded separately, and an average output calculated for each laser. Knowing laser power outputs and pixel dwell-time allows the estimation of the photon energy delivered per image pixel.

First, to approximate the number of photons per unit time per unit area onto the imaging scene, it is required to consider laser scanning speed and beam diameter (or spot size) on the target for determining laser dwell time:

$$\text{Dwell time (s)} = \frac{\text{laser spot size (mm)}}{\text{scan speed (mm s}^{-1}\text{)}} \qquad \text{(S1)}$$

In this study, actual pixel dimensions are dependent on scanned area dimensions (i.e., 900 x 900 mm, at 1000 lines: each pixel measures 0.81mm^2). As each line is recorded serially for a set sampling frequency, the observed signal corresponds to the area directly under the laser spot, the diameter being in the order of 1.5 to 2 mm depending on the laser used. A laser spot size larger than pixel diameter signifies a certain degree of oversampling. Additionally, any other type of stray light such as daylight and artificial light sources, water column bioluminescence and scattering would also be integrated into the recorded signal if it were not rejected via selective bandpass filters. In the current imaging conditions, stray light was controlled for by working in a light proof laboratory and eliminating artificial light sources.

Continuous wave laser line scan photon budget

To calculate the number of photons per unit time and unit area, we must initially calculate the energy per photon (E_{ph}) per laser wavelength used, where:

Power (P) = laser diode output in W
Wavelength (**λ**) = laser diode wavelength in m (e.g., 450 nm = 450E^{-9} m)
Planck's constant (h) = 6.6261E^{-34} J s
Speed of light (c) = 2.9989108E^{8} m·s^{-1}

$$E_{\text{ph (laser wavelength)}} = \frac{hc}{\lambda} \text{ (or hv)} \qquad \text{(S2)}$$

$$\mathrm{E_{ph\ (laser\ wavelength)}} = \frac{6.6261\mathrm{E}^{-34}\ \mathrm{J\ s} \times 2.9979\mathrm{E}^{8}\mathrm{m\ s}^{-1}}{\mathrm{laser\ wavelength\ E}^{-9}\ \mathrm{m}} = \mathrm{nb\ Joules\ per\ photon} \tag{S3}$$

This gives the energy in Joules per photon, where 1 Joule is the work required for producing 1 watt of power for 1 second (1 J = 1W s)

The definition of photon flux can be described as the number of photons per second per unit area, and does not consider the photon energy:

$$\text{Photon flux } (\Phi) = \frac{\text{nb photons}}{\text{per second per unit area}} \tag{S4}$$

Since the number of photons per Joule can be found by calculating the inverse of Joules per photon, then:

$$\text{Photons per second}_{\ laser\ \lambda} = \frac{1}{\mathrm{E_{ph\ (laser\ wavelength)}}} \times \text{ laser power}_{\text{in W at laser } \lambda} \tag{S5}$$

For example, a laser diode operating at 450 nm and 0.08 W:

$$\text{Photons per second}_{\ laser\ \lambda} = \frac{450E^{-9}\ m}{(6.6261\mathrm{E}^{-34}\ \mathrm{J\ s})\ (2.9979\mathrm{E}^{8}\ \mathrm{m\ s}^{-1})}\ \ 0.080\ \mathrm{W} \tag{S6}$$

$$\text{Photon flux (i.e., Photons per second}_{\ 450nm\ at\ 0.08W}) = 1.81229\mathrm{E}^{17} \text{ photons per second}$$

Finally, irradiance on the pixel is dependent on imaged substrate pixel dimension and dwell time.

Table S1. Multispectral laser serial imager components list

Imager components	Description
Laser diodes (CW)	
450nm	450nm Osram PLT5 450B, 89 mW output, spot size of 1.0 mm at 2.3m
488nm	490m Sharp 490nm GH04850B2G, 80 mW output, spot size of 1.2mm at 2.3m
520nm	520nm Osram PLT 520, 26.5 mW output, spot size 1.5mm at 2.3m
Analog to Digital Converter	
National Instruments USB-6366	8 AI (16-Bit, 2 MS/s), 2 AO (3.33 MS/s), 24 DIO USB Multifunction I/O Device
Photodetector (PMT)	
Hamamatsu R9880U-20	Wavelength (Short, Long, Peak): 230 nm, 920 nm, 630 nm
	Anode Gain Typ. $2.0E^6$
	Anode Dark Current (after 30 min.) Typ. 10 nA
	Anode Dark Current (after 30 min.) Max. 100 nA
	Rise Time Typ. 0.57 ns
Dichroic beam combiners (emitter)	
427 nm	Cut-On: 427.00 nm; Transmission: 439 - 647.1 nm; Reflection: 372 – 415 nm
466 nm	Cut-On: 466.00 nm; Transmission: 473 - 647.1 nm; Reflection: 439 - 457.9 nm
503 nm	Cut-On: 503.00 nm; Transmission: 515.5 - 647.1 nm; Reflection: 473 – 491 nm
Filters (detector)	
450nm	10 nm full width half maximum (FWHM)
488nm	10 nm full width half maximum (FWHM)
520nm	10 nm full width half maximum (FWHM)
580nm	10 nm full width half maximum (FWHM)
685nm	15 nm full width half maximum (FWHM)
Scanner (galvo)	
FARO Nutfield 3XB	Variable field size (i.e., up to 1 m × 1 m), variable spot size (i.e., down to 30 um), variable scan rate, optional z-axis compensation for flat-field

Table S2. Approximate dwell time per 1 mm spatial displacement.

Laser diode (nm)	Spot diameter size (mm)	Spot area (mm^2)	Scanning speed (mm s-1)	Dwell time (s)
450	1.5	1.767	20000	0.000075
490	1.5	1.767	20000	0.000075
520	2.0	3.141	20000	0.000100

Table S3. Experimental tank-water inherent optical properties (Kd m^{-1}: downwelling attenuation coefficient; a m^{-1}: absorption coefficient; b m^{-1}: scattering coefficient) based on Solonenko and Mobley 2015 (2.3 m distance to target) and estimation for 490 nm.

IOP	475 nm	490 nm	500 nm	675 nm	685 nm	700 nm
Kd m^{-1}	0.0253	0.0291	0.0316	0.4450	0.5050	0.5950
a m^{-1}	0.0216	0.0256	0.0282	0.4350	0.4938	0.5820
b m^{-1}	0.0054	0.0049	0.0046	0.0021	0.0021	0.0020

Table S4. Estimated photon budget values per laser diode during experimental conditions.

Laser diode (nm)	Measured wavelength (nm)	Laser output power (W)	Joules per photon	Photons per Joule	Photons per mm^2 for dwell time
450	450.0	0.0800	$4.41431E^{-19}$	$2.26536E^{18}$	$7.69159E^{12}$ (0.000075s)
490	491.8	0.0800	$4.03912E^{-19}$	$2.47579E^{18}$	$8.40606E^{12}$ (0.000075s)
520	515.8	0.0265	$3.85111E^{-19}$	$2.59666E^{18}$	$2.19034E^{12}$ (0.000100s)