Wide Field-of-View Fluorescence Imaging of Coral Reefs

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Supplementary Note 1: Image Formation Model

Color sensing in a single sensor camera is done by placing three types of color filters over the pixels in the sensor, usually arranged in a matrix (*Bayer pattern*). Then, image intensities are linearly related to the object radiance at a pixel $\mathbf{x}$ weighted by the camera sensitivity at each wavelength [50,51]:

$$I_{\mathrm{c}}(\mathbf{x}) = k(\mathbf{x}) \int_{\Lambda} z_{\mathrm{c}}(\lambda) \left[L(\lambda, \mathbf{x}) T(\lambda, \mathbf{x}) + \mathrm{BS}(\lambda, \mathbf{x}) \right] d\lambda \quad , \tag{1}$$

where $\mathrm{c} = R, G, B$ is the color channel, and λ is a wavelength in the range of the camera sensitivity Λ. The scale $k(\mathbf{x})$ encompasses colorless intensity changes. Here $z_{\mathrm{c}}(\lambda)$ is the color channel sensitivity, and $L(\lambda, \mathbf{x})$ is the radiance of the object point imaged at that pixel, originating either from reflectance or fluorescence, in units of $[\frac{\mathrm{W}}{\mathrm{m}^2 \mathrm{sr}^{-1}}]$.

The term $T(\lambda, \mathbf{x})$ depicts the light attenuation by the medium ($T = 1$ in vacuum). In a scattering medium such as water, light that is scattered back by the medium to the camera contributes an additive backscatter $\mathrm{BS}(\lambda, \mathbf{x})$. In addition, the radiance $L(\lambda, \mathbf{x})$ is attenuated exponentially as a function of the wavelength dependent attenuation coefficient $\beta(\lambda)$ and the distance $D(\mathbf{x})$ of the camera from the object point imaged at the pixel $\mathbf{x}$:

$$T(\lambda, \mathbf{x}) = \exp\left[-\beta(\lambda) D(\mathbf{x})\right] \quad . \tag{2}$$

The scale $k(\mathbf{x})$ differs for each pixel and encompasses colorless intensity changes. It depends on the effective f-number, lens transfer function, viewing angle, and the angle between the projected ray to the optical axis [50]. From now on we omit the $\mathbf{x}$ notation for simplicity, and assume $k(\mathbf{x})$ has been calibrated.

During nighttime, the only contribution to image intensity is ideally from fluorescence F_{strobes}, but in reality there is also reflectance R_{leakage} caused by some non-zero overlap between the excitation and the barrier filter. Thus, the image intensity is

$$I_{\text{night}} = F_{\text{strobes}} + R_{\text{leakage}} \quad . \tag{3}$$

The excitation spectrum $s_{\text{excitation}}$ is defined as the relative efficiency of different wavelengths of incident light at exciting fluorescence and is related to the absorption spectrum of the fluorescent molecule. The emission spectrum s_{emission} is defined as the relative intensity emitted as a function of wavelength in units of $\left[\frac{1}{\text{nm}}\right]$, and is independent of the actual wavelengths used for excitation, as long as they are within the excitation spectrum [52,53]. The excitation irradiance $p_{\text{excitation}}\left[\frac{\text{W}}{\text{m}^2}\right]$ depends on the excitation spectrum of the fluorescent material $s_{\text{excitation}}\left[\frac{1}{\text{nm}}\right]$ by [52]

$$p_{\text{excitation}} = \int_{\Lambda} I_{\text{source}}(\lambda) s_{\text{excitation}}(\lambda) d\lambda \quad , \tag{4}$$

where $I_{\text{source}}(\lambda)\left[\frac{\text{W}}{\text{m}^2}\right]$ is the irradiance from the excitation source. This irradiance at an object point depends on the distance of the light source to the object R_{source}. In addition to attenuation by the water, free space propagation creates a $1/R^2_{\mathrm{source}}$ irradiance falloff. Hence [51]

$$I_{\mathrm{source}}(\lambda) = \frac{\exp\left[-\beta(\lambda) R_{\mathrm{source}}\right]}{R^2_{\mathrm{source}}} Q \cos\theta_{\mathrm{i}} \ , \tag{5}$$

where Q expresses the non-uniformity of the scene irradiance, caused by the anisotropy of the illumination. The $\cos\theta_{\mathrm{i}}$ term is a foreshortening factor, as the exposed surface area decreases as the angle between the surface normal and illumination direction increases.

Then, the fluorescence radiance is

$$L_{\mathrm{fluorescence}}(\lambda) = p_{\mathrm{excitation}} s_{\mathrm{emission}}(\lambda) \frac{\alpha}{\pi} \ \ , \tag{6}$$

where α indicates the fluorescence efficiency that depends on the concentration and type of the fluorophores in the object. The term $\frac{1}{\pi}$ assumes the fluorescence emission is isotropic [54]. Following Eqs. (1) and (6), the fluorescence intensity of color channel c is given by

$$F^{\mathrm{c}}_{\mathrm{strobes}} = p_{\mathrm{excitation}} \frac{\alpha}{\pi} \int_{\Lambda} z_{\mathrm{c}}(\lambda) s_{\mathrm{emission}}(\lambda) T(\lambda) d\lambda \ \ . \tag{7}$$

Note that there is no backscatter in the fluorescence image as it is eliminated by the barrier filter.

The radiance of light *reflected* from an object point illuminated by a point light source towards the camera [50] is expressed by

$$L(\lambda) = I_{\mathrm{source}}(\lambda) b(\lambda, \boldsymbol{\theta}) \ \ , \tag{8}$$

where $b(\lambda, \boldsymbol{\theta})$ is the bi-directional reflectance function at this object point, and $\boldsymbol{\theta} = (\theta_{\mathrm{i}}, \phi_{\mathrm{i}}, \theta_{\mathrm{r}}, \phi_{\mathrm{r}})$, are incident and viewing directions in spherical angles relative to a local coordinate system defined by the surface normal. Then, following Eqs. (1,8)

$$R_{\text{leakage}} = \int_{\Lambda} z_{\text{c}}(\lambda) \left[I_{\text{source}}(\lambda) b(\lambda, \boldsymbol{\theta}) T(\lambda) + \text{BS}(\lambda) \right] d\lambda \quad . \tag{9}$$

For a complete derivation of the backscatter term, $\text{BS}(\lambda)$, see [51].

In a linear camera, image intensities are related to spectrometer measurements via equation (1), where the spectrometer measures a scaled version of $L(\lambda)$

$$I_{\text{spectrometer}}(\lambda) = k_{\text{spectrometer}} L(\lambda) T(\lambda) \quad . \tag{10}$$

Here $k_{\text{spectrometer}}$ accounts for the integration area, numerical aperture and exposure time of the spectrometer. equation (10) assumes that the radiance is isotropic, which is valid for fluorescence emissions [54] and diffuse reflectances.

Supplementary Note 2: Ambient Light Subtraction Under Noise

During daytime, the color intensity recorded at a pixel is composed of two independent measurements: signal I_{ambient} from the ambient illumination and fluorescence F_{strobes} excited by the blue strobes:

$$I_{\text{day}} = F_{\text{strobes}} + I_{\text{ambient}} \quad . \tag{11}$$

The signal from the ambient light contains reflectance of the ambient light and fluorescence excited by the short wavelengths in the ambient illumination:

$$I_{\text{ambient}} = F_{\text{ambient}} + R_{\text{ambient}} \quad . \tag{12}$$

For a discussion of the relative intensities of F_{ambient} and R_{ambient} see Mazel (2003) [55]. When I_{ambient} is measured (for example, by imaging the same scene with the blue strobes turned off), equation (11) can be inverted to reveal the pure fluorescence signal:

$$F_{\mathrm{strobes}} = I_{\mathrm{day}} - I_{\mathrm{ambient}} \quad . \tag{13}$$

This is the *ambient light subtraction* method.

Here we examine what is the minimum ratio between strobe-induced fluorescence and contribution from ambient light, $F_{\mathrm{relative}} = F_{\mathrm{strobes}}/I_{\mathrm{ambient}}$ to yield a meaningful result for the ambient light subtraction, equation (13). The subtraction in equation (13) yields a meaningful signal only if the signal-to-noise-ratio (SNR) in F_{strobes} is above a certain minimum. The SNR is defined as [56]

$$\mathrm{SNR} = F_{\mathrm{strobes}}/\sigma_{F_{\mathrm{strobes}}} \quad , \tag{14}$$

where $\sigma_{F_{\mathrm{strobes}}}$ is the standard deviation (STD) of the noise in F_{strobes}. Denoting α as the minimum required SNR for visibility, the condition [56] for a meaningful signal in equation (13) is

$$F_{\mathrm{strobes}}/\sigma_{F_{\mathrm{strobes}}} > \alpha \quad . \tag{15}$$

The standard deviation of the noise in digital images can be modelled as the sum of a signal independent component and a signal dependent component (shot noise) [56]

$$\sigma^2 = \kappa^2 + I/\rho \quad , \tag{16}$$

where κ^2 encompasses the variance of the signal-independent components of the gray-level noise (amplifier readout noise, quantization, and dark current noise). The term ρ has the units of [electrons/gray levels] and represents the sensor gain, the number of photo-generated electrons required to change a value of a pixel by a unit gray level. The value of ρ depends on the quantum efficiency of the camera, the ISO setting within the camera and M, the maximum gray level value. For our cameras we measured $\rho = [0.35, 0.065]$ for ISO $= [160, 640]$ in the Canon 5DII, with $M = 65535$, using the method in Treibitz and Schechner [56].

In well exposed images the signal independent component is negligible relative to the photon noise ($\kappa^2 \ll I/\rho$), such that

$$\sigma^2 \approx I/\rho \quad . \tag{17}$$

The measurements I_{ambient} and I_{day} are statistically independent. Then, in a first order approximation, the noise variance of F_{strobes} from equation (13) is given by

$$\sigma^2_{F_{\text{strobes}}} = \sigma^2_{I_{\text{day}}} + \sigma^2_{I_{\text{ambient}}} \quad . \tag{18}$$

Combining Eqs. (15,17,18) yields the condition

$$(F_{\text{strobes}})^2 > (I_{\text{day}} + I_{\text{ambient}})\alpha^2/\rho \quad . \tag{19}$$

Then, combining Eqs. (13,19) yields a quadratic inequality in F_{relative}

$$\frac{\rho R}{\alpha^2} F^2_{\text{relative}} - F_{\text{relative}} - 2 > 0 \quad . \tag{20}$$

Taking the positive solution yields the condition

$$F_{\text{relative}} > \frac{\alpha^2 + \sqrt{\alpha^2 + 8\rho I_{\text{ambient}}}}{2\rho I_{\text{ambient}}} \quad . \tag{21}$$

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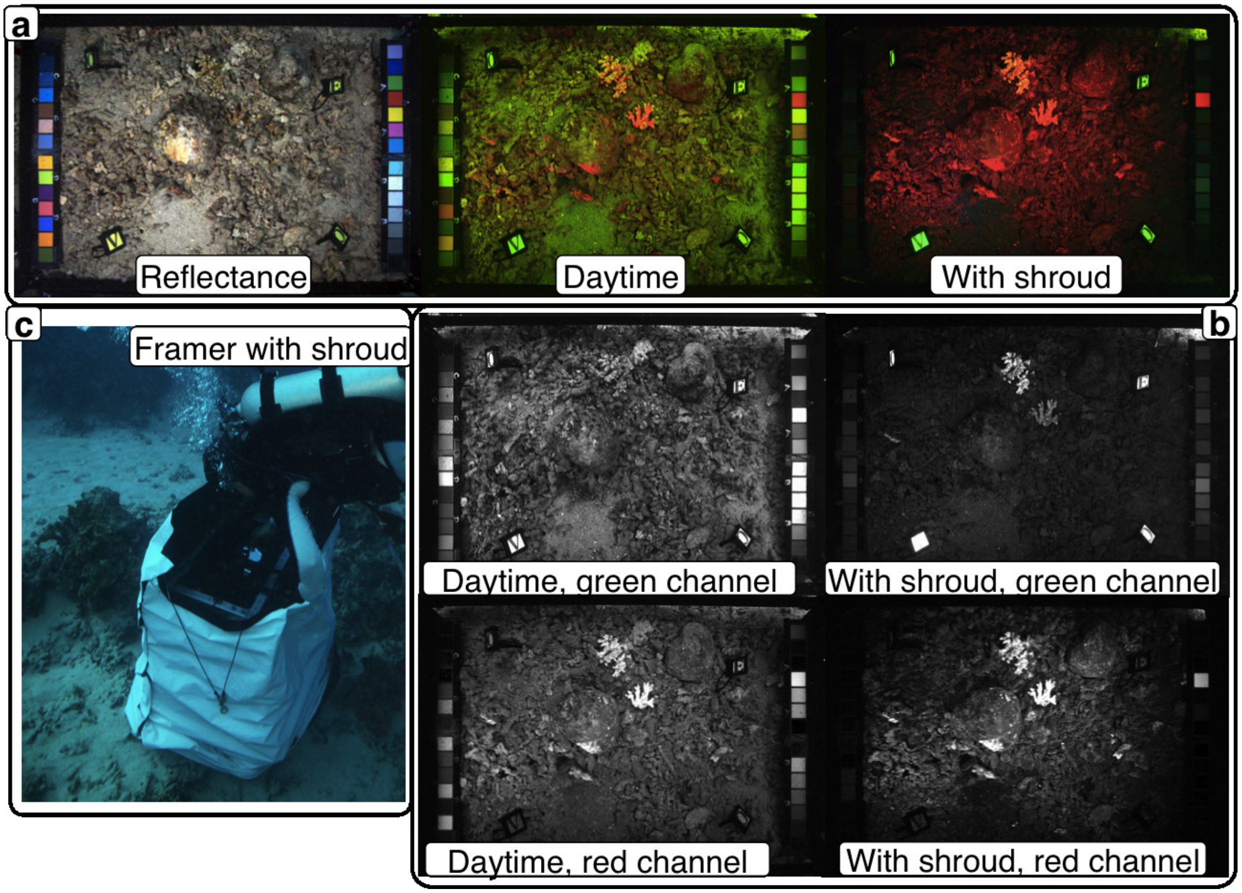
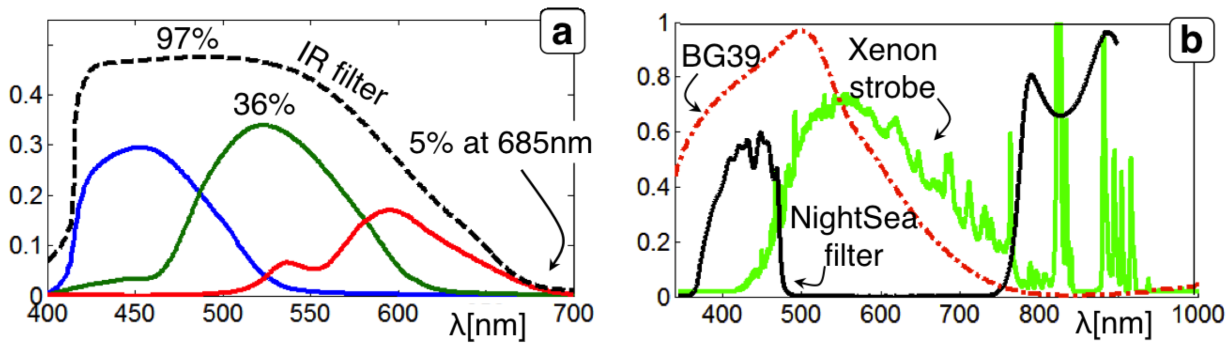
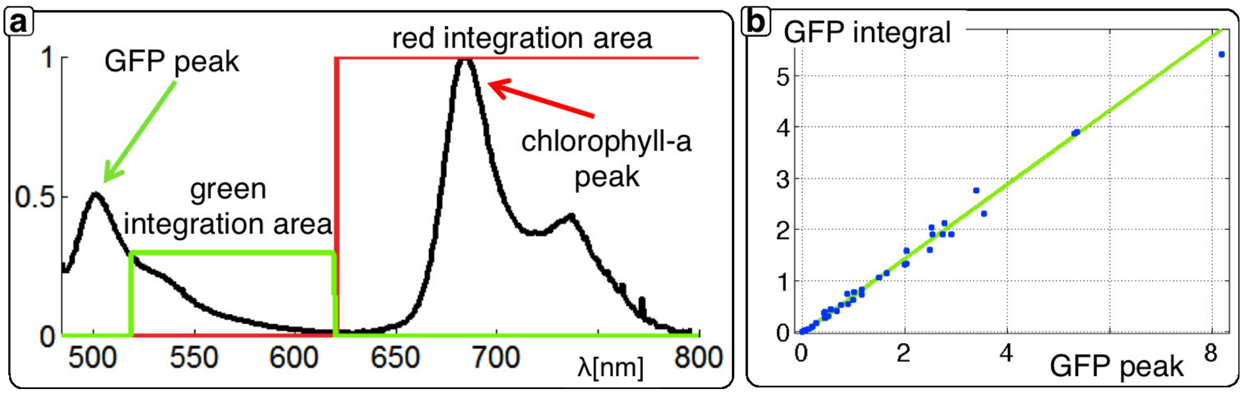


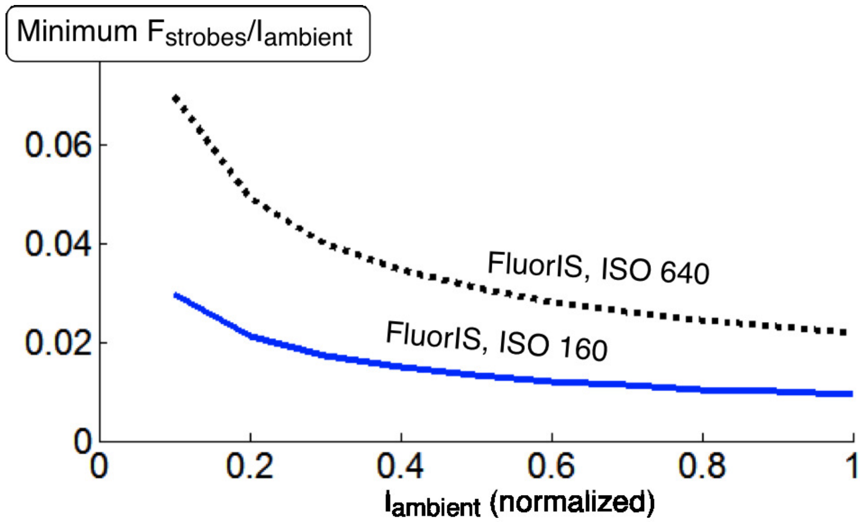
Supplementary Figure 1: **Imaging with a shroud during daytime.** For daytime fluorescence imaging it is also possible to mount a black fabric shroud around the framer. We used Ultra Bounce black grid cloth (Matthews Studio Equipment, California, USA) to cover the framer. The black side was facing inside to avoid light reflections. Velcro was used to fasten the fabric to the framer, to prevent light leakage from outside. We achieved darkness, as if the scene was imaged at night. Diving, moving and deploying the fabric is feasible in calm environments, but impractical in environments with strong surge and currents. All images were taken with the Fluorescence Imaging System (FluorIS) in Moorea, French Polynesia. (a) Comparison of a reflectance image (taken with white light), daytime fluorescence image, daytime fluorescence image taken with a shroud (left to right). (b) Comparison of the green and red channel of images taken with and without a shroud shows that the shroud blocked the ambient light reflectance. (c) Imaging with the shroud *in-situ*.



Supplementary Figure 2: (a) Camera sensitivity responses of the Canon 5DII with the mounted IR filter, and the transmission of an IR filter mounted on the sensor. The IR filter starts attenuating at approximately 570nm, and has significant attenuation above 650nm. At 685nm, the peak chlorophyll fluorescence, its transmission is only 5%. (b) Spectra of illumination components: strobes and strobe filters. The spectrum of the Xenon strobe is depicted in solid green. The blue Nightsea filter is depicted in solid black. Note that it attenuates most of the strobe's intensity. The blue filter blocks at least 10^{-2} of the light intensity between $486\text{nm} - 744\text{nm}$. However, it transmits IR wavelengths above 744nm, so just using the nightsea filter, the illumination is composed of blue wavelengths as well as undesired IR wavelengths. The glass filter BG39 (dashed red curve) attenuates the undesired long wavelengths above 744nm by more than 10^{-2}, with very small attenuation in the desired excitation wavelengths.



Supplementary Figure 3: (a) An example spectrum measured by the spectrometer, and the integration ranges used in equation (4). The green integration range starts from the barrier filter transmission and ends when the GFP emission is negligible. The barrier filter cuts the GFP peak out, as it is close to the excitation wavelength. Thus, integration of the GFP spectrometer data is on the long wavelength shoulder of the GFP signal. The red integration area spans the entire chlorophyll emission spectrum. (b) Values of the GFP emission from $n = 105$ measurements (relative units). The integral over the long wavelength shoulder of the GFP emission $\Lambda_{\mathrm{G}} = [520, 630]\mathrm{nm}$ is strongly correlated to the peak intensity ($r = 0.992$, $p < 0.001$, Spearman rank correlation coefficient), making the integral over Λ_{G} indicative of the peak intensity.



Supplementary Figure 4: **Limitations in daytime fluorescence imaging.** (a) Effect of bit depth and noise on the fluorescence signal recovery. (b) Recovering the strobe-excited fluorescence signal F_{strobes} during daytime (equation 13) is limited by noise levels. The fluorescence signal present in the image has to be above noise levels in order to be recovered. Here we depict the minimum recoverable fluorescence signal relative to the ambient light I_{ambient} intensity, as a function of I_{ambient}. In well exposed images (higher values of I_{ambient}), F_{strobes} can be 50 times lower, and still be detected. Higher ISO values yield higher noise levels, and thus for ISO 640, the minimum value of F_{strobes} is double that for ISO 160.