Wide Field-of-View Daytime Fluorescence Imaging of Coral Reefs

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***Abstract*—Coral reefs globally are experiencing rapid rates of decline associated with both local and global stressors. Improved monitoring tools are urgently needed to understand the changes that are occurring at appropriate temporal and spatial scales. Coral fluorescence imaging tools have the potential to improve both ecological and physiological assessments. Although fluorescence imaging is regularly used for laboratory studies of corals, it has not yet been used for large-scale *in situ* assessments. One of the obstacles to effective fluorescence surveying is the need for nighttime deployment, as reflectance from ambient light veils the fluorescence signal. In this paper we describe a method for effective daytime fluorescence imaging with an off-the-shelf camera. The method is based on subtracting an additional image of the ambient light from the daytime fluorescence image. This system enables wide field-of-view fluorophore surveying during the day, opening the possibility for extensive fluorescence surveys with consumer cameras. We also demonstrate the possibility of using a shroud to filter out sunlight in calm water.**

I. Introduction

Coral reef ecosystems are in a state of crisis, suffering massive global declines over the last three decades including up to 80% loss of coral coverage in the Caribbean [1] and 50% in the Indo-Pacific [2] and the Great Barrier Reef [3]. These declines are occurring rapidly, often at over 1% loss of coverage per year [2], [3], due to both local stresses such as pollution, overfishing and sedimentation as well as global climate change impacts such as global warming, ocean acidification and sea level rise [4], [5]. Thus, new non-invasive, rapid monitoring tools are urgently needed to better understand how coral physiology and reef ecosystems are responding to these stresses. Coral fluorescence imaging can complement standard underwater imaging for both aquaria and *in situ* monitoring of corals, but current fluorescence camera systems are of limited use for practical ecological surveys. A major methodological obstacle is the need for nighttime deployment in order to avoid signal contamination by ambient light (Fig. 1). However, nighttime deployments present risks to divers, and are more logistically complicated for underwater vehicles.

Fluorescence is defined as the reemission of photons with longer wavelengths than the absorbed photons [6]. In corals, two components mostly contribute to fluorescence. Photosynthetic pigments present in the symbiotic algae that live within the coral tissues contain chlorophyll-a that emits in the long red wavelengths (660nm-800nm). In addition, fluorescent proteins (FPs) in the coral animal tissue have emission peaks

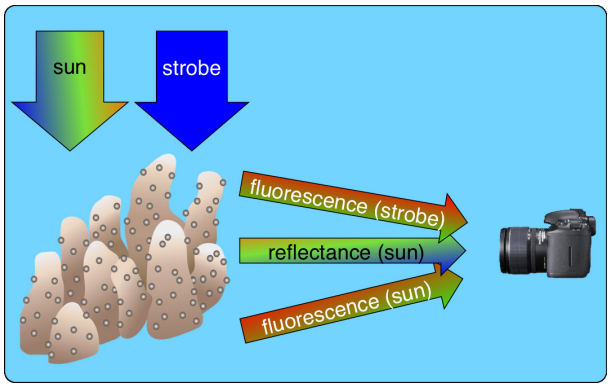

Fig. 1. **Daytime fluorescence imaging**. When imaging with a fluorescence setup during daytime, reflectance from ambient light contaminates the fluorescence signal. This signal mixture makes fluorescence imaging during nighttime preferable, but in many reef locations nighttime deployments are impractical.

in the range of 489nm-609nm (see [7] for a detailed list). Coral fluorescence plays an important role in coral studies. FPs can comprise up to 14% of the total protein content in some coral species [8], potentially contributing to important biological functions, some of which are not yet well defined. Changes in fluorescence can indicate heat stress [9] and be expressed as an early sign of coral bleaching prior to visible paling of the tissue [10]–[12]. Additionally, Green Fluorescent Proteins (GFPs) have been shown to play a role as light-induced electron donors, affecting photochemical reactions [13]. Measurements of chlorophyll-a fluorescence are often used to quantify photosynthetic ability, through pulse amplitude fluorometry (PAM) or through Fast Repetition Rate Fluorometry (FRRF) [14] and by estimating chlorophyll-a from photographs [15]. Over larger spatial scales, observations of coral fluorescence (both GFP and chlorophyll-a) can aid benthic cover classification [16], and contribute to identification of cryptic coral juveniles [17]–[20].

Previously, Mazel [21] recorded daytime coral fluorescence with a consumer camera by using very short exposure times (2 milliseconds). This reduces the input of ambient light and increases fluorescence intensity, as the strobe duration is similar to the exposure time. However, although this is the ideal solution for daytime fluorescence imaging, the number of consumer cameras able to achieve such short synchronization

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time is limited. To overcome this, imaging can be done at sunset or sunrise to decrease ambient light levels [17], [20], with limited operation time. In this work we present a method for daytime underwater wide-angle fluorescence imaging based on acquiring two frames of the same scene, one with the excitation strobes on, and the second with the strobes off. In addition, we show that in very calm water a shroud can be used to cover the camera yielding effective daytime fluorescence imaging using just a single image. We demonstrate effective fluorescence imaging during daytime, even at shallow depths in the presence of strong ambient illumination.

II. Imaging System

A fluorescence imaging system has three main components: 1) an excitation source emitting light only in wavelengths in the excitation range, 2) a camera with adequate sensitivity to detect the weak fluorescence signal, and 3) a barrier filter on the camera transmitting fluorescence emission while blocking the excitation illumination (Fig. 2a). Here we describe our considerations for choice of these components, and the specific components used for our system. Similar systems can be built from other components complying with the spectral and sensitivity considerations described below.

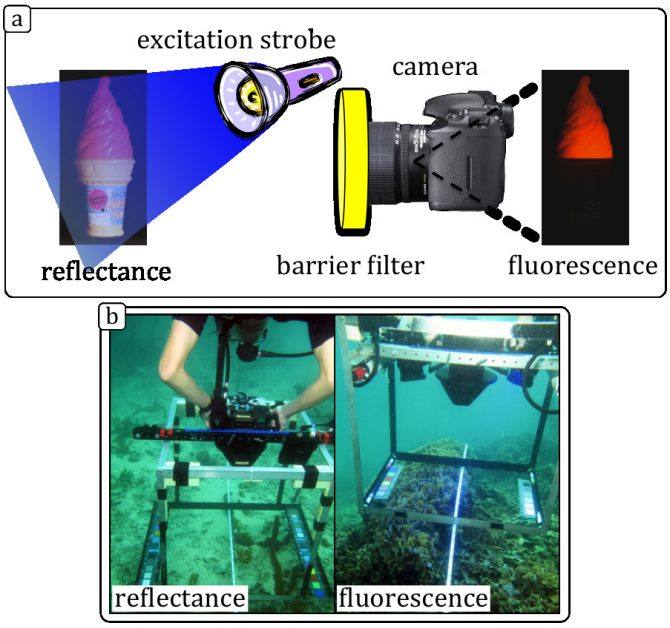

Fig. 2. **Fluorescence imaging setups.** a) A basic setup for fluorescence imaging. The excitation source emits short wavelength (typically UV/blue). A barrier filter is mounted on the camera to block the excitation spectrum. The plastic toy shown in the image is made of two parts, where only the upper cone fluoresces, and thus this is the only part that is visible in the fluorescence image on the right. b) Underwater deployment of our reflectance and fluorescence imaging setups (left and right, respectively). Both systems are mounted on a framer with similar dimensions, and image a wide field-of-view during daytime.

A. Camera

The sensitivity of consumer color cameras in the long visible wavelengths is very weak, as they are designed to imitate the the sensitivity of the human visual system, optimizing for a pleasing appearance. As CMOS and CCD sensors are sensitive to long wavelengths, this is achieved by including an infrared (IR) filter on top of the color filters. The standard IR filter cuts out wavelengths above ~ 650nm, minimizing sensitivity to the desired chlorophyll-a fluorescence. Therefore, we modified the camera by removing the IR filter and replacing it with a filter that transmits the entire spectrum. The modification was done by LifePixel inc. (www.lifepixel.com), using their "full spectrum" option. Such conversions have been done previously for the astronomy community, to image IR [22], and also for photographers for artistic reasons. Fig. 3 demonstrates the benefit of using a modified camera for chlorophyll-a fluorescence imaging.

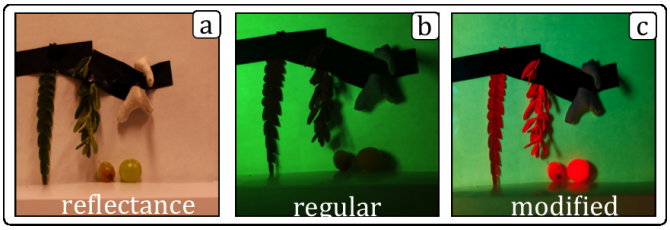

Fig. 3. **The benefit of using a modified camera for chlorophyll-a fluorescence imaging.** a) Reflectance image of a scene containing leaves and grapes, that contain chlorophyll-a. b) A fluorescence image of the same scene taken with a standard camera. c) The same scene imaged with a modified camera. The red signal from the chlorophyll-a fluorescence in the leaves and the grapes is much stronger.

This results in a camera with an increased sensitivity, particularly in the long wavelengths. Such a modification can be done on most consumer cameras. Specifically, we used the Canon 5DII camera for acquiring reflectance images and a modified Canon 5DII for fluorescence images. Both were used with wide angle lenses (Canon 17-40mm or Sigma 20mm). The cameras were housed in a Canon 5DII Sea&Sea housing with the Fisheye Dome Port 240 and a 40mm Sea&Sea extension ring for better alignment of the dome port with the lenses. For fluorescence, the barrier filter was a Tiffen $\#12$ yellow filter mounted on the lens. The camera was mounted 70cm from the target, achieving a wide field-of-view of $50\text{cm} \times 70\text{cm}$ (Fig. 2b).

B. Illumination

We used a few models of off-the-shelf Xenon strobes. For reflectance imaging we used Ikelite DS-161 (160Ws) strobes (one or two strobes). For fluorescence imaging we used two Sea&Sea YS-250 (250Ws) strobes and two Inon Z-240 (240Ws) strobes. These two models are the strongest commercial underwater strobes currently available, and also have a fast recharge rate. The strobes were positioned in the 4 corners around the camera. The strobes were attached with two strobe arms each, such that they were as close as possible

to the corals, while illuminating the entire field of view. This yielded good images with camera settings of f#8, and ISO 640.

For fluorescence imaging, blue NightSea filters were used to filter the strobes (www.nightsea.com). In the visible spectrum, these filters transmit only blue light, the desired excitation band. However, from our experiments, these filters transmit long IR wavelengths, which are normally blocked by the camera IR filter. Thus, since we have removed the camera IR filter, an additional Schott glass GB39 was mounted on the strobes to block these long IR wavelengths.

III. Daytime Fluorescence Imaging

A. Ambient Light Subtraction

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:

$$I_{\mathrm{day}} = F_{\mathrm{strobes}} + I_{\mathrm{ambient}} \quad . \tag{1}$$

Note that the signal from the ambient light contains reflectance of the ambient light and fluorescence excited by the short wavelengths in the ambient illumination. For a discussion of the relative intensities of reflectance and fluorescence stemming from ambient light see Mazel (2003) [23]. When I_{ambient} is measured (for example, by imaging the same scene with the blue strobes turned off), Eq. (1) can be inverted to reveal the pure fluorescence signal:

$$F_{\mathrm{strobes}} = I_{\mathrm{day}} - I_{\mathrm{ambient}} \quad . \tag{2}$$

We term this method *ambient light subtraction*. In practice, the two images I_{day} and I_{ambient} should be acquired with the same camera settings (ISO, aperture, shutter speed, focus) and with minimal delay, so they can be aligned with minimal motion between images, and to avoid changes in ambient illumination such as clouds and wave caustics.

Figs. 4,5 demonstrate this method on images taken in shallow reefs during daytime in Bocas Del Toro, Panama, and in Eilat, Israel. In both, the fluorescence signal is clearly visible in the subtracted frame. In Fig. 4, the left coral has higher levels of chlorophyll-a as opposed to the right coral. In Fig. 5, the fluorescence signal is barely noticeable in the original image, as it was taken at a depth of 2m and has a significant amount of ambient light. In the difference image, the fluorescence is visible in the two big corals and many small fragments.

Our method assumes that the camera response is linear, a common case for raw format images. The system's linearity was verified by imaging an Xrite ColorChecker chart at six exposures. For raw conversion we used Dcraw open source code (www.cybercom.net/~dcoffin/dcraw) and Matlab.

B. Using a Shroud

It is also possible to mount a black fabric shroud around the framer for daytime fluorescence imaging. To test the feasibility of this method 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. While diving the shroud was rolled up and tied with bungee cords, and once the frame was set on the imaged scene, we rolled the shroud down and used velcro to firmly attach it to the framer to avoid light penetration from the sides. Diving, moving and deploying the fabric is feasible in calm environments, but impractical in environments with strong surge and currents. In sufficiently calm conditions, the shroud efficiently blocked the ambient light, as demonstrated in Fig. 6.

IV. Discussion

In this paper we demonstrated wide field-of-view, high sensitivity daytime fluorescence *in situ* imaging of coral ecosystems using off-the-shelf components. This method will enable expanded physiological and ecological research applications utilizing *in vivo* fluorescence, addressing pressing issues in coral physiology, ecology, and conservation.

We have shown that fluorescence can be extracted from pairs of registered images, where in one of them the strobes are off. A key for the success of this method was the modification of the camera for increased sensitivity. In the future we plan to build a device that can automatically control the strobes to acquire the image pair, without the need for manual control of the strobes. The quality of the results depends on the noise levels in the images, and we plan to analyze that in the future.

Acknowledgments

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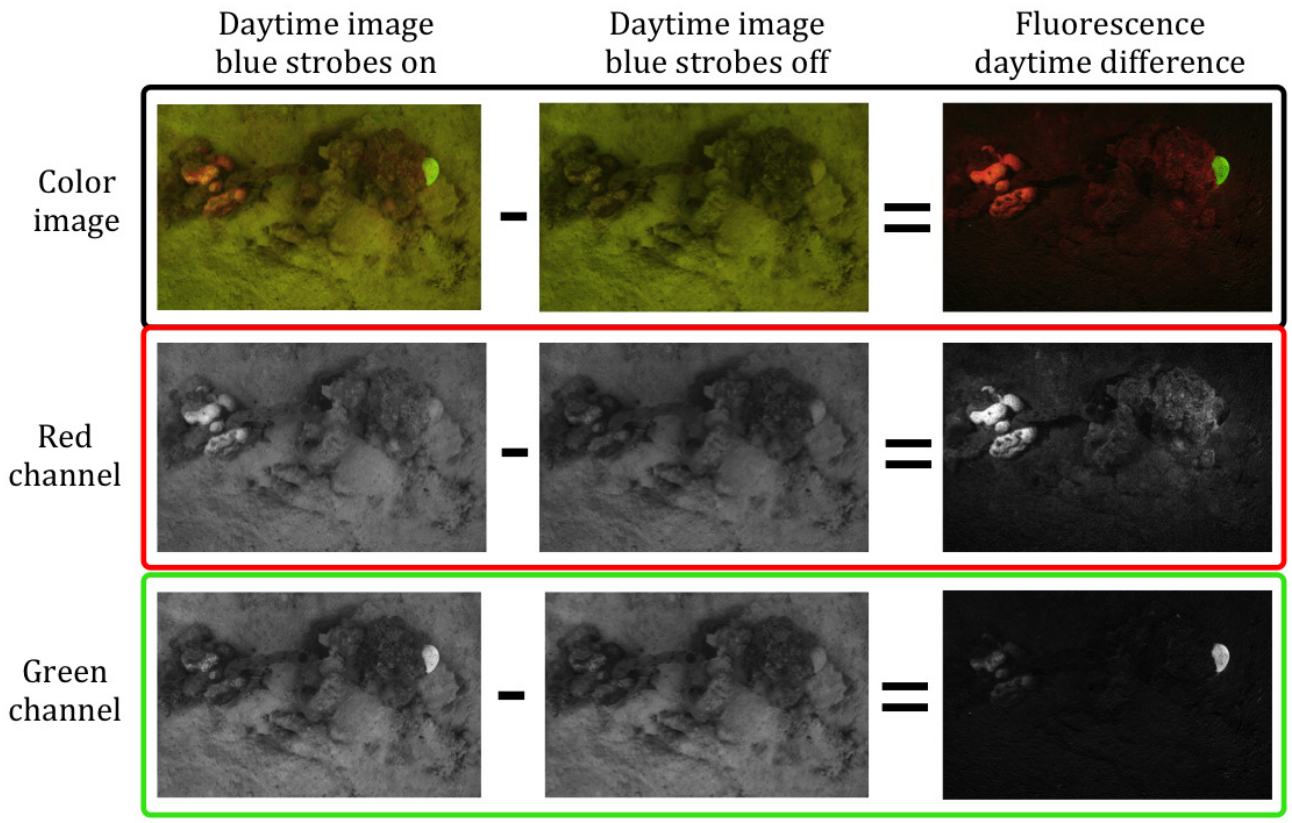

Fig. 4. **Ambient light subtraction.** When imaging fluorescence during daytime, subtraction of the image that contains only ambient light from an image taken with the blue strobes on produces a fluorescence image (Eq. 2). Images were taken in Bocas Del Toro, Panama at a depth of 5m. The shutter speed was 1/250s, and the ISO was set 640.

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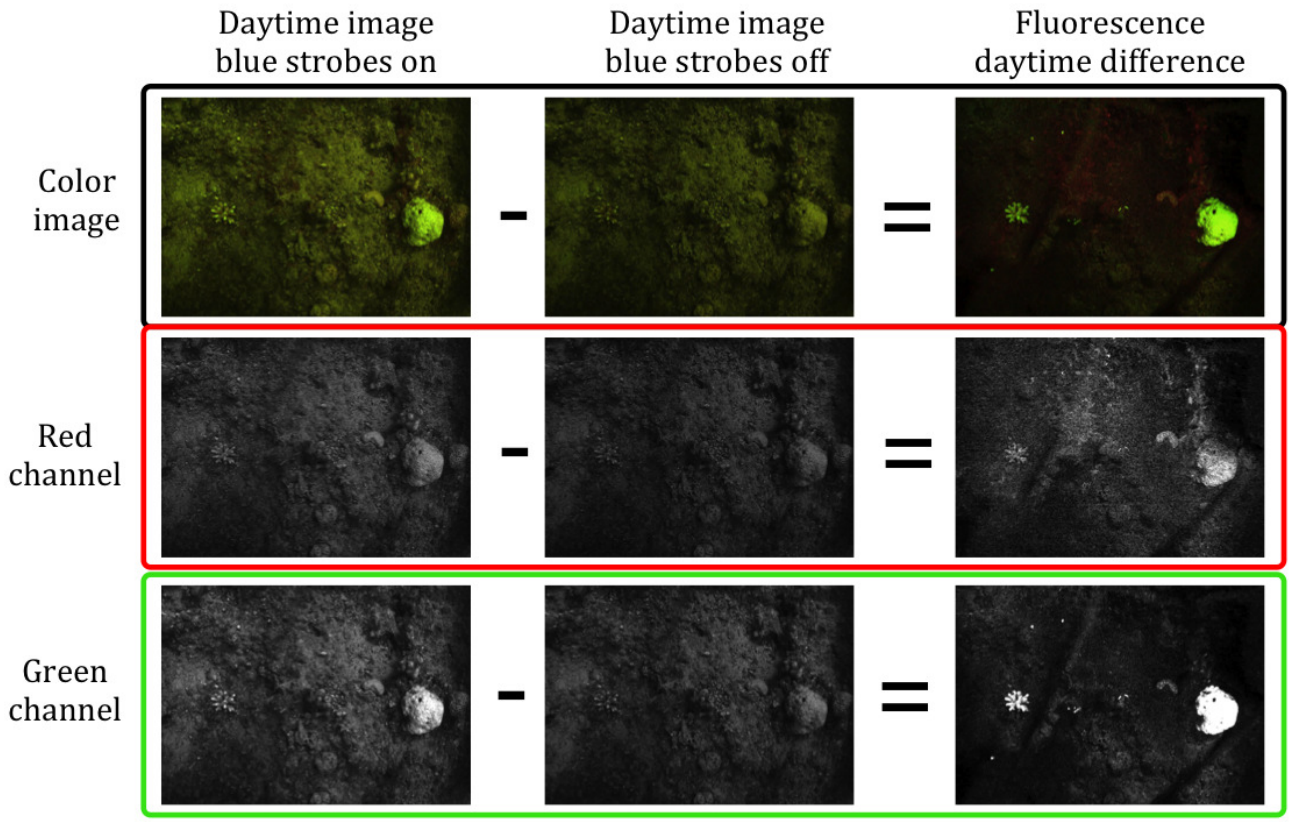

Fig. 5. **Ambient light subtraction.** Results of applying Eq. (2) on images taken at a depth of 2m in Eilat, Israel. Images taken by Gal Eyal and Jonathan Shaked.

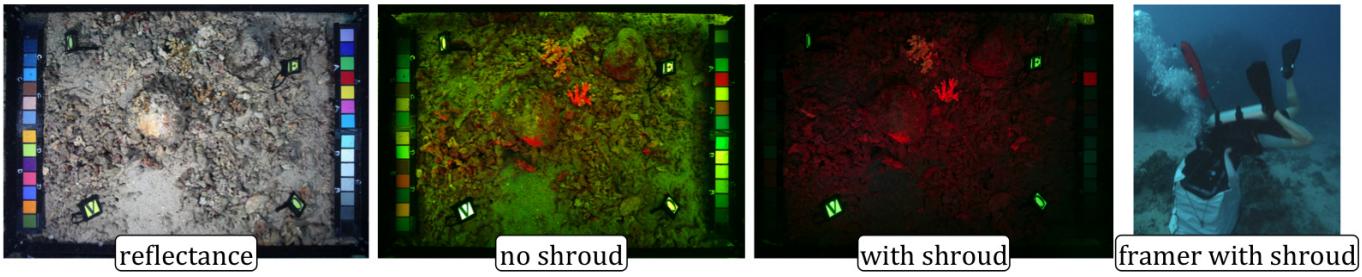

Fig. 6. **Daytime fluorescence imaging using a shroud to eliminate ambient light.**