

## **Underwater Fluorescence Reference Panels**

Charles Mazel  
Physical Sciences Inc.  
20 New England Business Center  
Andover, MA 01810  
Phone: (978) 983-2217 Fax: (978) 689-3232 E-mail: [mazel@psicorp.com](mailto:mazel@psicorp.com)  
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### **LONG-TERM GOAL**

This work is undertaken as part of a long-term effort to understand the nature of fluorescence in the marine environment. The emphasis is on fluorescence imaging and detection of targets of interest, both biological and man-made.

### **OBJECTIVES**

The objective of this effort is to develop Fluorescence Reference Panels (FRP's) for use in the field and in the laboratory with the Fluorescence Imaging Laser Line Scan (FILLS) system operated by the Electro-Optics Identification group of the Coastal Systems Station (CSS), Panama City, and with other in-water sensing systems. The FRP's are intended to serve as calibration surfaces for characterization of system performance and for development of data processing algorithms. To meet these needs the FRP's should: fluoresce at the appropriate wavelengths; have a range of fluorescent yields that bracket those expected in the natural environment; have fluorescence properties that will be stable over time and with exposure to underwater ambient daylight; and be amenable to routine handling in the laboratory, on shipboard, and by divers for underwater installation.

### **APPROACH**

We are incorporating stable fluorescent dyes in an acrylic polymer matrix. Acrylic is well known to be stable in seawater, and is often used for viewports, underwater housings, aquarium windows, etc. The choice of acrylic allows us to address the other mechanical requirements in a straightforward manner. Furthermore, Dennis Pacheco of Physical Sciences has extensive experience in incorporating fluorescent dyes in plastics for other applications.

Achieving the desired optical properties for this project was a multistep process. We first identified several candidate dyes for each of the FILLS detection channels. We then had test panels fabricated using each dye at two different loading concentrations. We also experimented with different levels of non-fluorescent pigments to achieve a mid-range reflectance. The fluorescence excitation/emission and reflectance properties of each panel were then measured quantitatively. We developed a mathematical figure-of-merit that enabled us to relate the fluorescence of the panels to the fluorescence of natural targets. The figure-of-merit is the product of the relative excitation level at the FILLS laser wavelength of 488 nm, the fluorescence efficiency of the surface, and the percent of the emitted signal that falls within the FILLS channel detection bandwidth. The goal was to match the figure-of-merit of the FRP's to the figure-of-merit of natural targets.

# Report Documentation Page

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The data from the first set of test panels was used to specify a smaller set of second generation test panels. This included test panels with single fluorescent dyes and panels that incorporated a mixture of two dyes. These panels were analyzed in the same fashion as the first set. The results were fine-tuned to specify the dye mixtures and concentrations for the panels that would be used in the field.

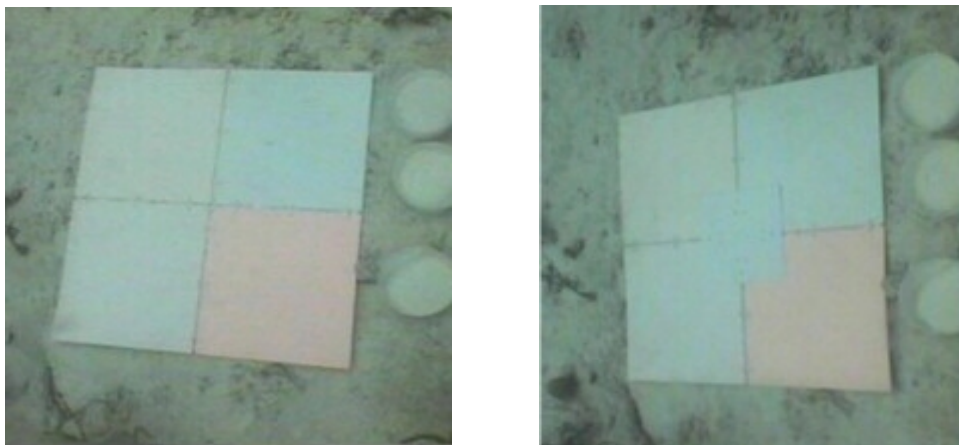
## **WORK COMPLETED**

We successfully went through the iterations to produce panels that we felt would be suitable for field use. Two different fluorescent dye combinations were selected, and each of these was incorporated in the acrylic matrix at two different concentrations to produce low and high levels of fluorescence. We made six 2' x 2' panels of each of these four formulations. In addition we made four non-fluorescent panels that incorporated the same level of non-fluorescent pigment as the others, but no fluorescent dye. One face of each panel had a matte finish, with the aim of producing a diffuse reflectance, while the other side was smooth.

We designed and manufactured aluminum crossbar frames so that the four different fluorescent panels could be assembled into a large square. The mounting system was designed so that the panel matrix could be assembled underwater by divers, as a 4' x 4' square would be unwieldy to handle in the water column.

The panels and frames were completed in time for use during the May/June 1999 Coastal Benthic Optical Properties (CoBOP) field campaign at Lee Stocking Island (LSI), Bahamas. The panels were deployed in a depth of approximately 9 meters in the area known as Rainbow Gardens. Due to operational problems with the FILLS system the panels were only imaged with one fluorescence channel during the field exercise.

At the end of the CoBOP field campaign we used a smaller square of the non-fluorescent material to mask a corner of each of the deployed panels (Figure 1). We left the panel matrix in place to determine the long-term resistance of the panels to photobleaching under actual field conditions. The intention is to view and measure the fluorescence properties of the panels when we return to LSI in January of 2000.

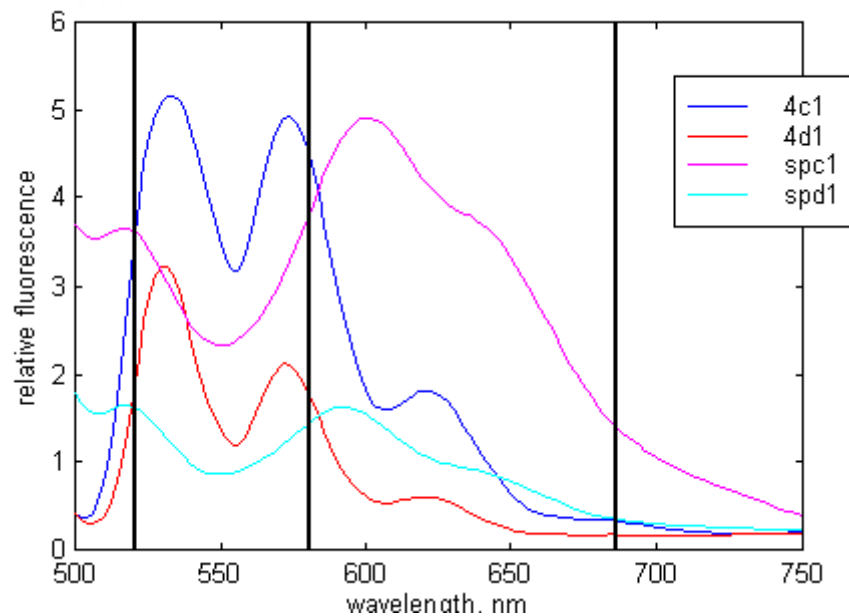


*Figure 1. Frame-grabbed video images of the underwater fluorescence reference panel matrix before (left) and after (right) installation of the non-fluorescent mask.*

The remaining panels were delivered to CSS for use in conjunction with other field exercises or for system testing under laboratory conditions. One set of panels was used in conjunction with a FILLS exercise conducted off Key West, Florida, in the summer of 1999.

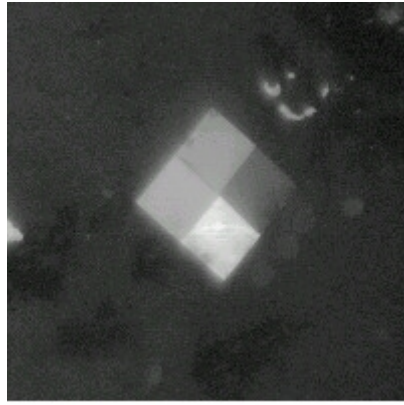
## RESULTS

The panels appear to meet the desired specifications, although further testing will be necessary under more controlled conditions with the FILLS system. Figure 2 shows the fluorescence emission spectra for the four panels manufactured for field use. Panels *4c1* and *4d1* contained different concentrations of the same single dye, while panels *spc1* and *spd1* contained two dyes in two different concentrations. All four panels show measurable output in the FILLS green (520 nm) and orange (580 nm) detection bands, while only panel *spc1* shows significant response in the red (685 nm) band.



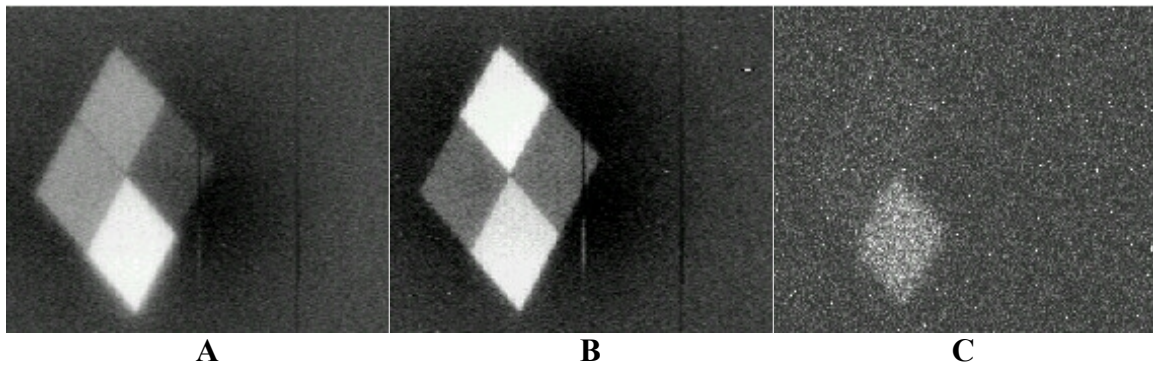
***Figure 2. Fluorescence emission spectra of the FRP's manufactured for field use. Black vertical lines indicate the approximate center of the FILLS detection bands at 520, 580, and 685 nm.***

Figure 3 is a FILLS image of the panels as deployed at LSI. Only the green detection channel was functioning when this image was made. The fluorescence above and to the right of the panels arises from a coral. Note that the fluorescence intensities of the panels appear to bracket that of the target, as desired.



**Figure 3.** *FILLS image, green fluorescence channel. Clockwise from the top of the diamond pattern, the panels are 4c1, 4d1, spc1, and spd1.*

Figure 4 are FILLS images of the panels as deployed at Key West. The panels are in the same orientation as in Figure 3. The green channel image (A) is similar to that of Figure 3. The orange channel image (B) indicates that panel 4c1 has the greatest response, followed by panel spc1. Only panel spc1 responds in the red (C), as would be expected from the fluorescence emission spectra.



**Figure 4.** *Images of the FRP's made with the FILLS green (A), orange (B), and red (C) fluorescence channels.*

## **IMPACT/APPLICATION**

These highly uniform fluorescent panels will be used for calibration of the FILLS system both in the laboratory and in the field. During the next CoBOP field campaign identical panel sets will be placed at several different depths along the FILLS transect line, so that they will be imaged from different stand-off distances. This will provide data needed to test algorithms that are being developed to correct the FILLS raw data for factors such as water path attenuation of the outgoing laser beam and the emitted fluorescence.

## **TRANSITIONS**

The Ocean Optics group at the University of South Florida has borrowed a set of panels for use with their ROV-mounted spectroradiometric and multispectral imaging systems. The intended use is

similar to that with the FILLS system – equipment performance verification, calibration, and algorithm development.

Alan Decho, a collaborating CoBOP researcher, is using small pieces of this uniform fluorescent material as a substrate for biofilms. He is making measurements to investigate the influence of the films on the optical properties of surfaces.

## **RELATED PROJECTS**

We have been investigating the fluorescence characteristics of seafloor substrates and organisms as part of the CoBOP program. The data collected through that work produced the knowledge of fluorescence characteristics of naturally occurring targets that was needed to specify the dye loading for the FRP's.