(Editors Note: Technology Forum is intended to stimulate thought and discussion on diverse oceanographic technology issues. We welcome contributions on technological issues relative to ocean science, but particularly to U.S. GLOBEC.)

3-D Bioluminescence Mapping

by Edith A. Widder

An intensified video transect technique has been used to identify and map bioluminescent organisms based on the spatial and temporal patterns of their stimulated bioluminescent displays (Greene et al., 1992; Widder et al., 1989, 1992).

This technique evolved as a by-product of an investigation of unstimululated background bioluminescence levels in the Monterey Canyon. The intensified video transect technique employed during that investigation, using the single person submersible DEEP ROVER, was originally designed to measure the abundance of potential luminescent sources in the water column. During horizontal transects, a video recording was made of bioluminescent displays from organisms which were mechanically stimulated to luminesce as they contacted a 1-meter diameter screen mounted in front of the submersible. An automated computer image-analysis program was then used to count the number of sources stimulated during the transect. During the course of this investigation, it became apparent that it was frequently possible to identify the sources of these displays, often to the species level, based on the temporal and spatial patterns of their bioluminescent displays (Widder et al., 1989). In this initial investigation, identified displays were limited to gelatinous sources. More recently the technique has been adapted for use on the JOHNSON-SEA-LINK submersible (Figure 1) and was employed to map the micro-scale distribution patterns of the euphausiid *Meganyctiphanes norvegica*. Simultaneous estimates of krill abundance and patchiness were made with a dual-beam acoustic method. Comparison of abundance and patchiness estimates made with these two very different mapping techniques demonstrated no significant differences between these estimates (Greene et al., 1992). In addition, the bioluminescence technique simultaneously mapped a population of co-occurring ctenophores (Widder et al., 1992).

Data Analysis

Through a collaboration with MRJ, INC., image recognition algorithms are now being developed that will automate the identification of organisms based on their bioluminescent displays, and then map their locations in three-dimensional space. Using parallel processing, the video frames from a bioluminescence transect are stacked, one on top of each other, to create a solid volume. With this data format the high-contrast video images can then be thresholded and the background made transparent so that only the bioluminescent events are visible in three-dimensional space. This volume data structure is used to identify luminous events and extract features such as intensity; duration; size; kinetics (rise time, decay rate, pulsing rate, if any); release of extracellular material if any (glowing particles, scintillating particles, diffuse clouds); and the coordinates of the point of impact. This information is then sent to the luminous object data structure, where it is stored and organized into flash categories. Densities of different flash categories are then calculated from the volume scanned. Distances to nearest neighbors, etc. can then be calculated.

Advantages

1) Bioluminescence mapping is both a high-frequency and high-resolution technique which samples a statistically significant volume. Using the species-specific label of bioluminescence, dinoflagellates as small as 50 µm can be identified in real-time in a field of view of one meter. Therefore, multiple bioluminescent species of sizes extending from 50 µm to 1 m can be mapped simultaneously with a single video camera.

2) Bioluminescence recordings are very high contrast, thus edge detection is much less of a problem compared to illuminated video recording. As a result, the algorithms for categorization and identification of bioluminescent signatures are extremely simple compared to image recognition algorithms, which require much higher resolution images and must deal with issues like multiple

(Cont. on page 11)

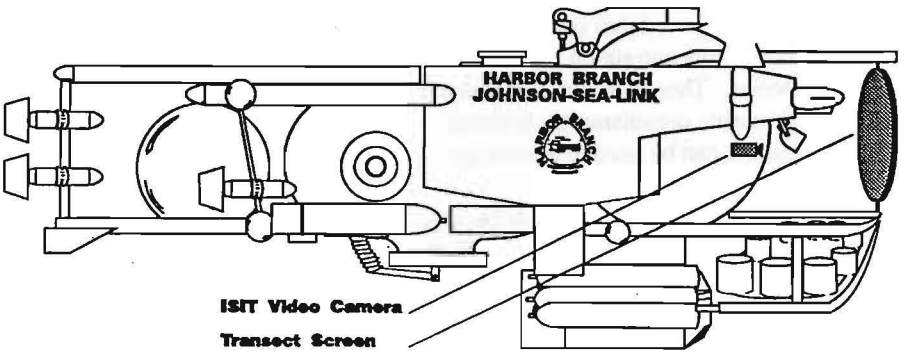

Figure 1. Configuration of the JOHNSON-SEA-LINK submersible for making intensified video recordings of stimulated bioluminescence during horizontal transects.

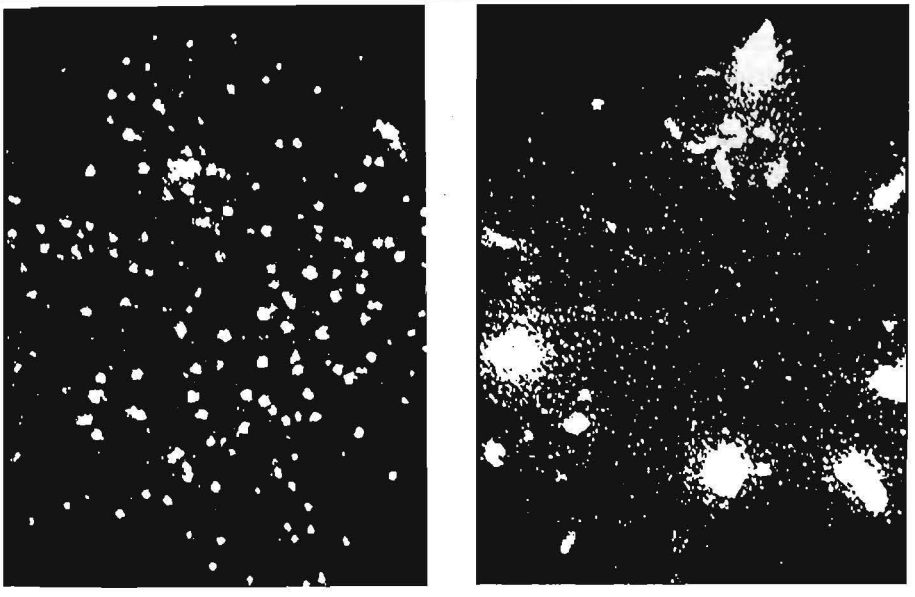

Figure 2. Examples of bioluminescence mapping.

Left Image. Bioluminescent flashes recorded in a dinoflagellate layer.

Right Image. Bioluminescent flashes recorded in a ctenophore layer. The figure-8 at the top of the image is characteristic of the lobate ctenophore Bolinopsis infundibulum. The other bright displays are extracellular emissions characteristic of the cydippid ctenophore Euplokamis sp. The field of view in these images is 0.74 m by 1.0 m.

Bioluminescence—(Cont. from page 6)

possible orientations of specimens and the identification of types and numbers of appendages.

Disadvantages

1) Not all marine organisms are bioluminescent. However, many of the dominant species in the ocean are light producers and virtually every cubic meter of the ocean contains some bioluminescent organisms. These include all pelagic krill as well as many species of copepods, dinoflagellates, ostracods, larvaceans, amphipods, mysids, decapods, polychaetes, fish, squid and most of the gelatinous zooplankton. Due to the enormous complexity of the marine environment, it has been suggested that investigators should concentrate on a few key species. Therefore, the fact that not all marine organisms are bioluminescent can be used to advantage.

2) Bioluminescence in some taxa, esp. dinoflagellates, is inhibited by high-light intensities and the majority of bioluminescent zooplankton undergo vertical migration, occupying surface waters only at night. Furthermore, bioluminescent displays provide the greatest contrast and are thus most easily detected when it is dark. For these reasons, three-dimensional mapping of near-surface waters of the oceans is done only at night or at dusk and dawn while following migrating layers.

3) Some mechanical disturbance is required to stimulate bioluminescence. However, because of the slow transect speed (0.6 kt) and the large mesh size of the screen (1800 μm), the pressure wave in front of the screen is small, as evidenced by the lack of screen avoidance by krill (Widder et al., 1992).

4) Definitive identification of a particular organism with a particular bioluminescent display must be based on *in situ* measurements, since displays from captured specimens are often radically different from those recorded *in situ*. Therefore a dual camera system, which records high-resolution images of organisms superimposed on their bioluminescent displays (Widder, 1992) is being adapted for *in situ* work.

(Edith Widder is a marine scientist at the Harbor Branch Oceanographic Institution).

References

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