Bioluminescence Truth Data Measurement and Signature Detection

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LONG-TERM GOALS

To determine how low light phenomena, both bioluminescence and solar radiation below 200 meters, influence the distribution and behavior of marine organisms.

OBJECTIVES

The immediate objective is to address the issue of how to generate more and better measurements of bioluminescence to meet increased operational needs of Naval planners. We have recently upgraded the design of the High Intake Defined Excitation Bathyphotometer (HIDEX-BP) (Widder et al., 1993), which is the US Navy standard for bioluminescence measurements (Bivens et al., 2001). This new system, referred to as HIDEX II (Widder et al., 2003), maintains all of the original HIDEX I design principles but has been upgraded with an ancillary sensor suite to reflect current technology and Windows compatible software. HIDEX II has been delivered to the Naval Oceanographic Office and is now in use. We are now developing HIDEX III, which will incorporate the newest optical and electronics technology, expand the envelope of information that can be derived from the instrument and is intended as a test bed for the calibration of the increasing number of small bathyphotometers that are being developed for use on ROVS, AUVS, Gliders, and moorings.

APPROACH

Since the Naval Oceanographic Office wants all bioluminescence measurements referenced to HIDEX, the HIDEX III platform is being designed so that additional BP's may be attached to the platform for simultaneous, side-by-side *in situ* comparisons. For these comparisons to be meaningful it is necessary to know what organisms are responsible for the bioluminescence potential being measured. This is essential because although many small BPs may demonstrate good correlations with HIDEX measurements in a dinoflagellate-dominated environment, they may deviate significantly in a zooplankton-dominated environment, due to these swimmers' avoidance of the inlet. To address this issue HIDEX III is being designed to optimize characterization of the bioluminescence potential to facilitate the identification of which organisms are responsible for the measured light emissions. This information is essential for modeling efforts, as any attempt at forecasting bioluminescence potential must account for the behavior of the organisms responsible (e.g. photosynthetic or non-photosynthetic, vertical migrators or not, rheotactic or not). The design for HIDEX III is being developed collaboratively by myself, Dr Eran Fuchs and HBOI Engineering.

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In order to have adequate testing capabilities for HIDEX III, as well as for the various small BPs that are to be characterized, biological calibrations must be conducted in a test tank that has a sufficiently controlled environment to ensure darkness and temperature control and that incorporates a large enough reservoir of filtered sea water to permit the controlled introduction of cultured bioluminescent organisms. This new laboratory, known as the Marine Biophotonics Facility is under the direction of Dr. Eran Fuchs.

For bioluminescence measurements to be both operationally and ecologically meaningful it is essential that we develop a transfer function between BP measurements and the bioluminescent signature that will result from a defined stimulus in a particular body of water. To these ends we are collaborating with Drs Mike Latz (Scripps) and Jim Rohr (SPAWAR Systems Center San Diego) to test how bioluminescent signatures change with different species and concentrations and how these changes in signature relate to measurements made with different bathyphotometer designs. We are also collaborating with Dr. Sonke Johnsen (Duke University) to mathematically model the radiative transfer of these signatures. The goal is to correlate BP measurements with detectability.

WORK COMPLETED

The Marine Biophotonics Facility is now complete and includes the unique combination of a fully equipped optical laboratory in combination with a 2500-gallon modular test tank, an articulating crane and an advanced filtration system for ocean and freshwater applications (Figure 1). The tank and crane can accommodate instruments up to 4 m long and 500 kg in weight. The facility is fully light tight and temperature controlled and can handle all phases of design, testing and optical and biological calibrations.

Figure 1. HIDEX II being readied for calibration in the 2500-gallon test tank in the newly completed Marine Biophotonics Center. The HIDEX II is supported by the articulating crane and the inlet is coupled to a filtration baffle that insures that none of the bioluminescent dinoflagellates used in the biological calibrations are recycled through the detection chamber.

The design phase for HIDEX III is nearly complete. The fiber optics used in the first two generations will be replaced with an array of independent photomultiplier tube modules. The output from this array will be used to calculate both total light and the population e-fold factor. Additionally, we hope

that by introducing a much higher sampling rate (10 KHz vs.2 Hz) we will be able to extract information about organism concentrations and species identification. To this end we will incorporate the HIDEX III into the Vertical Instrument Platform (VIP) which will include a SPLAT CAM (Widder, 2002) for identification and 3D mapping of bioluminescent displays and a high speed plankton sampler for plankton identifications as well as an array of ancillary sensors (CTD, fluorometer, altimeter, Wetlabs AC-9 absorption and attenuation meter, Wetlabs C-Star transmissometer and a Wetlabs Light Scattering Sensor (LSS), and HBOI Low Light Auto-Radiometer (LoLAR). Data from these systems will be correlated with the HIDEX III array data to develop the counting and identification programs.

The photomultiplier tubes (PMT's) have been selected and are being tested. A completely new design of the instrument's sample chamber, which assures optimization of the optical sensors without changing the hydrodynamics, has been completed. The new design incorporates an on-board computer, which is a step towards operating the instrument in new modes of operation, such as a moored tethered system that can collect data over extended periods of time. A careful cost/benefit analysis was carried out to determine whether the PMTs should be housed individually or in a single housing. Individual housings were selected. As a consequence the light collection packages can function as stand-alone instruments with applications well beyond this system (Figure 2).

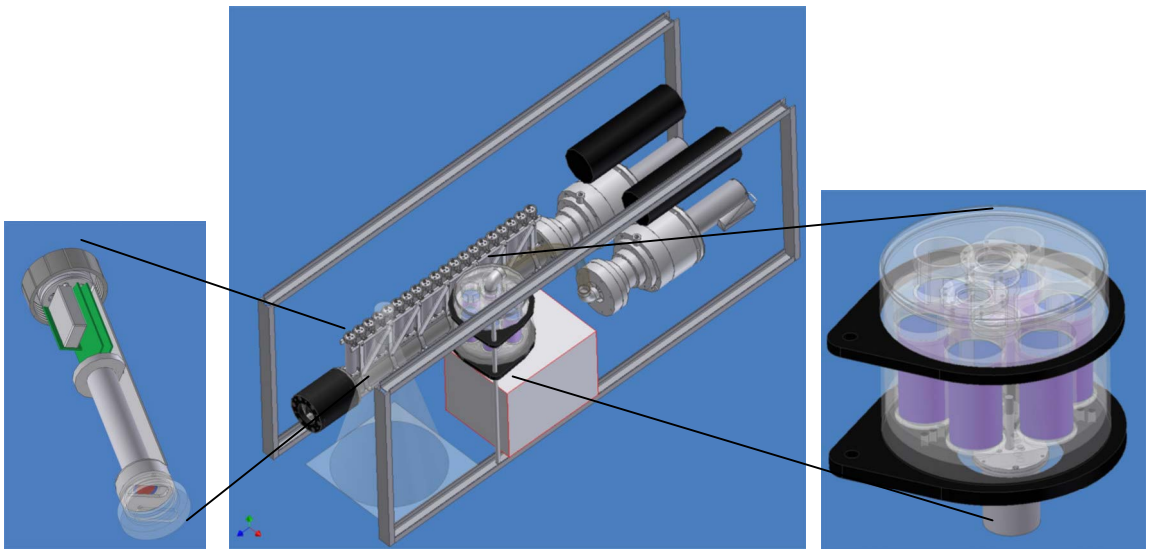

Figure 2. Graphic showing planned layout of Vertical Instrument Profiler. The detection chamber is mounted horizontally with the inlet at the left and the pump at the right. 20 individually housed PMTs are mounted down the length of the detection chamber. Blow-up drawing shows cylindrical design of one of the PMT housings. Mounted to one side of the inlet is the SPLAT CAM with the camera viewing a SPLAT screen mounted on the base of the VIP. To the right of the SPLAT CAM is the high-speed suction sampler. Blow-up drawing shows 6 cylindrical plankton-sampling cartridges mounted in a carousel. To the right of the plankton sampler is its high-speed pump, identical in design to that used behind the HIDEX III detection chamber. Shown above the pumps are 2 black cylinders representing the ancillary sensors.

The collaboration with Latz and Rohr for signature detection studies is under way. The bioluminescence signature apparatus, which consists of a turbulent jet in a vertical tank within a 2 foot diameter integrating sphere has been built. Three agitators will be used to correlate the measured total

photon flux generated by the hydrodynamically defined turbulent jet with measurements of "bioluminescence potential." These agitators are 1) the NAVO BIOLITE agitator 2) a bathyphotometer developed by Losee and Lapota and a low-flow agitator (0.1 L/s vs. 0.2 L/s for the other two) developed by Latz and Rohr. These three agitators will be tested against HIDEX in the new Biophotonics Facility using dinoflagellates with different flash characteristics.
The collaboration with Johnsen is also well under way. Using radiative transfer software and Monte Carlo and Fourier methods, the modeling of the energy and image transfer of bioluminescent signals has been successfully completed. The model can predict the brightness and appearance of a bioluminescent signal as a function of distance, water type, and the spatial acuity of the detector. The propagation of images under different values of these parameters is currently being prepared for publication in *Limnology and Oceanography*.

RESULTS

A detailed performance analysis of the new instrument predicts at least an order of magnitude higher sensitivity of the new design in comparison with previous generations. By eliminating fiber-optic light collection and converting entirely to a "sensor at the site" arrangement, we now expect to detect a signal as low as a single *Protoperidinium* flash (2 $X10^{10}$ p/s). In addition the temporal resolution will be improved by several orders of magnitude resulting in far better resolution for measurements of flash kinetics.

IMPACT/APPLICATIONS

Because of increased operational needs of Naval planners for accurate bioluminescence detection and prediction, there is a critical need for a greatly expanded database of bioluminescence measurements from the world's oceans. To be operationally useful these data must be accurate. Specifically, measurements made by one set of instruments must be directly comparable to those made by another. The HIDEX III/VIP will provide a test bed for field characterization of the increasing number of small bathyphotometers that are being developed for use on ROVS, AUVS, Gliders, and moorings, while the new Marine Biophotonics Facility provides a unique calibration laboratory for accurate bioluminescence calibrations of these detectors. The HIDEX III/VIP will also encompass in a single platform, data collection capabilities that have in the past required major field efforts and multiple platforms and will therefore represent a significant advance in the data available for modeling efforts. Modeling of bioluminescence signature detection will also be much improved by the direct measurements of signature total photon flux relative to "bioluminescence potential" measurements being undertaken in collaboration with Latz and Rohr and by the signature radiative transfer model being developed in collaboration with Johnsen.

TRANSITIONS

The Biophotonics Laboratory is now fully operational and available for testing of bathyphotometers. The HIDEX II was tested here prior to delivery to NAVOCEANO.

RELATED PROJECTS

With support from NSF we are currently developing the latest generation of Low Light Auto-Radiometer (LoLAR), which is the most sensitive *in situ* radiometer available for measuring

downwelling irradiance. The LoLAR is being designed for use on a variety of platforms, including the VIP.

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