

“ It was as if the Milky Way, as seen through a telescope, scattered in millions like glittering dust, had dropped down into the ocean, and we were sailing through it. ”

— C. W. Thomas, 1877
Voyage of the Challenger 2:85

Michael Latz and Jim Rohr

GLOWING with the Flow

Ecology and Applications of Flow-Stimulated Bioluminescence

Imagine yourself on a boat in the open sea on a calm summer night. As the vessel rocks gently, you notice innumerable flashes of blue-green light along its sides. Intrigued, you dive in and become engulfed in a mantle of ethereal light. Kick your legs and it's as if a flashlight has been illuminated at your feet. In the distance, you notice large streaks of light moving just beneath the water's surface. Suddenly a particular streak rushes toward you at alarming speed. Luckily, it's only a dolphin, which passes by you in a pyrotechnic coat trailing filaments of light.

As fairy-like as this scene may seem, the phenomenon responsible for it isn't magic. It's bioluminescence—the emission of visible light by biological organisms. While people are familiar with the flashes of fireflies, they are less apt to realize that bioluminescence can also light up the ocean.

In fact, the emission spectrum of most bioluminescence is tuned for optimal transmission through oceanic waters: It has a peak in the blue-green range (470-490 nm) for plankton and is greener (500-520 nm) for bottom-living animals. Yellow and red bioluminescence is less common in the marine environment than it is



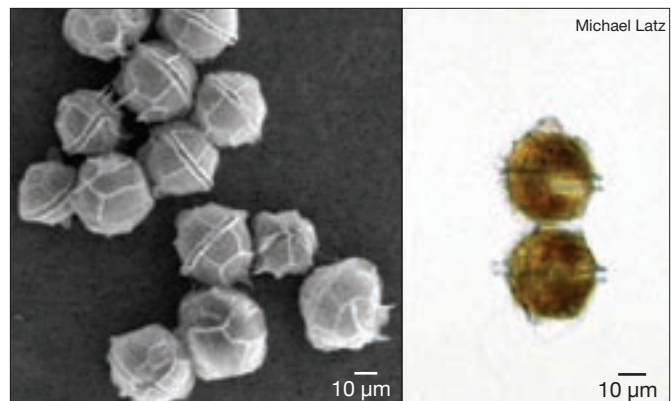
Bioluminescence stimulated by the motion of a kayaker in a bioluminescent bay in Puerto Rico. The bright display is due to the abundance of the dinoflagellate *Pyrodinium bahamense*.

Frank Llosa, www.biobay.com

in terrestrial ecosystems, because the optical transmission of air is not as wavelength-selective as water.

Often associated with water movement, bioluminescence was once thought to be due to friction between water and the salt within it. Later, Benjamin Franklin surmised that the light was caused by a large number of what he called “extremely small animalcules.” It was not until the 19th century that biologists such as G.A. Michaelis and C.G. Ehrenberg described and isolated microscopic organisms called dinoflagellates, which are now known to be the primary cause of bioluminescence near the sea surface.

Since then, bioluminescence has become well recognized as a tool that is used in varying degrees by many oceanic creatures, ranging from single-celled organisms to fish and even sharks. In nighttime surface waters or the perpetual darkness of the deep ocean, optical signaling is a common and effective form of



Cells of the dinoflagellate *Pyrodinium bahamense*. (Left) Scanning electron microscopy. (Right) Live cells using light microscopy.

Michael Latz



Dinoflagellate bioluminescence is an anti-predation strategy that can act as a burglar alarm. When a zooplankton preys on a dinoflagellate (*right*), the dinoflagellate is stimulated to flash, making the zooplankton visible to its fish predator. The result is that the normal feeding behavior of the zooplankton is disrupted and fewer dinoflagellates are eaten.

communication among organisms. Bioluminescence is used to attract prey (e.g., anglerfish), as a beacon to find prey (flashlight fish), as camouflage to avoid predators (some squid, shrimp and fish), as a deterrent to scare off predators (dinoflagellates, jellyfish and many others) or as a signal to potential mates (worms). Nighttime visual predators such as squid and fish use bioluminescent displays as a means of locating prey.

Bioluminescent light is produced from a chemical reaction involving the oxidation of a substrate called luciferin by the enzyme luciferase. There are different biochemical families of luciferins and luciferases, so those for fireflies are distinct from those of dinoflagellates, worms and fish, for example.

Bioluminescence through the ages

Accounts of oceanic bioluminescence extend back to ancient times. The Greek philosopher Anaximenes (500 B.C.) described the light produced from the sea when struck by an oar, while the Roman historian Titus Livius (59 B.C.E. – 17 C.E.) likened bioluminescence to fire when he claimed “the sea was aflame.” The Roman poet Martialis (100 C.E.) refers to a woman who:

“ ... plunged into the gleaming pool seeking to escape embrace. But the wave betrayed the lurking dame: brightly she showed, though covered by the overlapping water. ”

Since then, accounts of bioluminescence have been associated with the movements of dolphins, fish, divers and torpedoes.

Spotter planes have located fish schools by the bioluminescence they stimulate. World War II aviators have relied on the illuminated wakes of aircraft carriers to find their ship at night, while planes and ships used bioluminescence to locate and track enemy ships and submarines.

Dinoflagellate bioluminescence

In coastal regions, the primary source of flow-agitated bioluminescence is dinoflagellates. These single-celled organisms are common members of the plankton—tiny marine plants, animals or bacteria that float on or near the ocean’s surface. Bioluminescent dinoflagellates range in size from about 30 μm to 1 mm, and are found in all the world’s oceans. In some areas, such as the so-called bioluminescent bays of Puerto Rico, their concentrations are so high that they produce breathtaking bioluminescent displays throughout the year.

Laboratory experiments have provided insight into the role that dinoflagellate bioluminescence plays in marine ecology. Dinoflagellate flashes decrease the grazing rate of their predators by disrupting their feeding behavior and thus reducing the number of dinoflagellates consumed. Dinoflagellate bioluminescence is also thought to act as a “burglar alarm” to attract a secondary predator that threatens to eat the primary predator. When handled by a predator, the dinoflagellate cell is triggered to flash by the imparted mechanical stress.

Dinoflagellate bioluminescence is also stimulated by fluid shear stresses caused by waves, surge and the flow boundaries of moving animals and objects. The cellular signaling pathway that triggers bioluminescence is complex and only partially understood, but the luminescent chemistry is ultimately caused by a drop in pH due to an influx of protons within the cell. The time from stimulus to light emission is less than 20 ms, making it one of the most rapid

cellular processes known. Each dinoflagellate cell can produce more than one flash, which lasts on the order of 100 ms. However, the bioluminescence capacity of a cell is depleted once all its available luciferin has been oxidized.

Measuring bioluminescence in the digital age

Individual dinoflagellate flashes can be readily discerned by the dark-adapted human eye, with intensities ranging from 10^8 to 10^{10} photons/s/flash, depending on the species. Although “seeing is believing,” our eyes are not ideal sensors for evaluating bioluminescence intensity because visual sensitivity varies with dark adaptation, and color perception is lost under low light conditions when the more sensitive rod photoreceptors are used. Bioluminescence is best measured with specialized low light or intensified detectors with computer control and data acquisition.

The intensity of bright luminescent displays can be evaluated using a photodiode-based photometer. However, the detector of choice is an analog or photon-counting photomultiplier (PMT) for high sensitivity and fast response time. Because bioluminescence displays occur at fast time scales, scanning spectrometers are not used for measuring the emission spectrum. Rather, the spectral distribution of bioluminescence is measured by spectrographs coupled to intensified silicon diode or intensified charge coupled device (CCD) detectors.

Bright bioluminescence can be imaged in true color using consumer digital cameras and camcorders, but scientists typically use intensified CCD cameras, and, more recently, electron multiplication digital CCD cameras offering high gain, low noise and high bit resolution. These imaging strategies convert photons into electrons. The trade-off for enhanced sensitivity is that color information is lost; thus, patterns of bioluminescence are revealed in black and white.

Under field conditions, background illumination from stars and moonlight, as well as anthropogenic sources, limits researchers’ ability to observe bioluminescent events. Emerging multispectral imaging approaches could potentially be used to detect a dim bioluminescence signal among the brighter background, based on the narrow spectral distribution of bioluminescence compared to the broader spectrum of surrounding illumination. Even in the presence of “noise,” the bioluminescence signal can be boosted with a sensor with a target channel optimized for bioluminescence, and one or more guard channels at other wavelengths.

Flow-stimulated bioluminescence

Research into the flow stimulation of bioluminescence combines marine biology with experimental and computational fluid mechanics in order to identify the hydrodynamic features associated with the luminescent response. Flow-stimulated bioluminescence has been commonly associated with turbulent flow. However, experimental work with several independent flow fields reveals that the level of bioluminescence is related



Michael Latz stimulates bioluminescence of cultured dinoflagellates. True color image.

Scripps Institution of Oceanography, University of California, San Diego

to the level of fluid shear stress and volume of flow, and not particularly to the laminar or turbulent nature of the flow.

Using several different types of experimental flow fields, researchers have found that the minimum threshold for stimulation of bioluminescence occurs in flows with shear stress values of 0.02 – 0.3 N m^{-2} , depending on the species of dinoflagellate. At that threshold, only a small fraction of the population is stimulated. The shear levels in breaking waves and along most flow boundaries are above the response threshold, but typical levels of flow within the ocean’s interior are not. This range of sensitivity ensures that dinoflagellates’ capacity for bioluminescence would not be depleted under typical oceanic flow conditions. Experimental results are consistent with natural observations of bioluminescence caused by moving boundaries and wave and surge conditions.

Mapping bioluminescence of the world’s oceans

Bioluminescence represents an operational threat to U.S. Navy nighttime operations. Submarines and other covert vessels risk detection when they cause flow-stimulated light emission among naturally occurring plankton. This hazard is even more severe in shallow coastal areas, where photon sources are closer to the surface, and higher plankton abundance can result in brighter bioluminescence.

To identify high-risk areas, the Naval Oceanographic Office Laboratory Branch has been tasked with measuring the bioluminescence potential of the world’s oceans. It has amassed



Bioluminescence in a breaking beach wave during a red tide of the dinoflagellate *Lingulodinium polyedrum* in Santa Barbara, Calif. True color image.

thousands of hours of measurements. Researchers conduct bioluminescence surveys with bathyphotometers—sensor packages developed by Naval and academic research laboratories that couple a flow agitation chamber with a sensitive photometer, usually a photomultiplier detector. Bioluminescence is stimulated by intense flow agitation within a transparent chamber as seawater is pulled through. Oceanographic ships that map bioluminescence deploy bathyphotometers in the upper 100 to 200 m of the ocean.

The U.S. Navy standard for bioluminescence is the HIDEX bathyphotometer—a refrigerator-sized instrument developed at the University of California, Santa Barbara. HIDEX pumps seawater up to 44 L s^{-1} through an excitation grid to stimulate bioluminescence. It is best suited for open ocean regions, where

bioluminescence levels can be low. A towed version called TOWDEX continuously profiles the upper ocean while undulating behind the moving ship.

In coastal areas, where bioluminescence levels are typically higher, oceanographers can use low-volume bathyphotometers, which offer the advantages of smaller size, reduced weight, lower power requirements and ease of use. As underwater autonomous vehicles (UAVs) and unattended buoys become more common as measurement platforms, low-volume bathyphotometers will become the instruments of choice. The Multipurpose Bioluminescence Bathyphotometer (MBBP), also developed at the University of California, Santa Barbara, is designed to be used in UAVs and other low-profile sampling platforms. The MBBP uses the pump impeller, located upstream of the light-detection chamber, to provide flow agitation for bioluminescence stimulation.

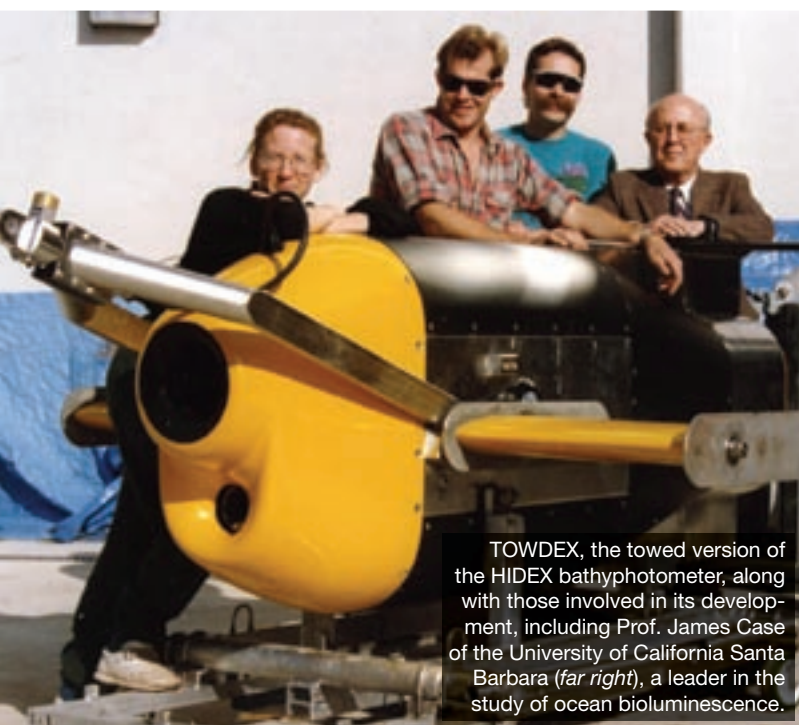
Researchers at Woods Hole Oceanographic Institution have developed a free-fall bathyphotometer, which is designed for either expendable or retrievable applications. It relies on its terminal velocity—rather than a pump—to force water through a grid to provide flow agitation. In addition, it uses a photodiode instead of a PMT as light detector. This new generation of small, less expensive bathyphotometers will greatly increase researchers' capability for conducting automated fine-scale bioluminescence sampling of the world's oceans.

Luminescent “snapshots” of fluid motion

To visualize many of the subtleties of fluid dynamics, Leonardo da Vinci would toss grass seeds into the flow of water and observe their trajectories. Five and a half centuries later, this work continues by using luminescent dinoflagellates as naturally occurring markers to derive insight into complicated flow fields.

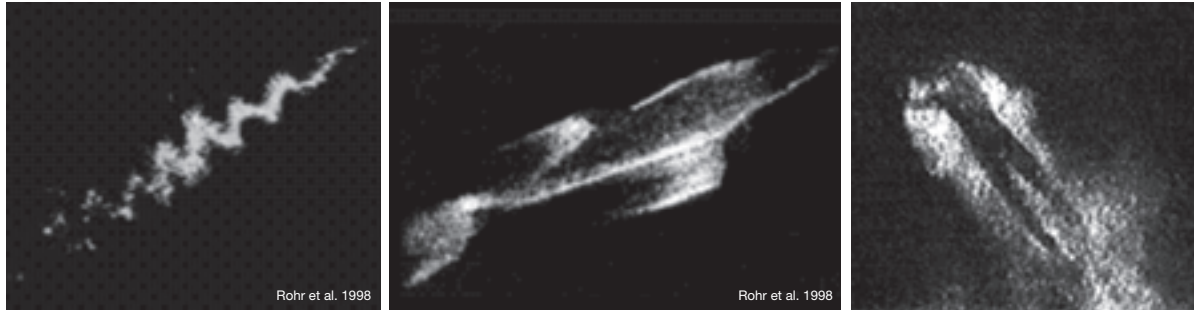
Laboratory studies provide a foundation for using dinoflagellate bioluminescence as a flow visualization tool. The organisms act as flow markers that emit light only when sufficiently high shear conditions are present. In coastal areas, dinoflagellate concentrations of $100 \text{ cells ml}^{-1}$ or greater are sufficient to highlight moving objects. In bioluminescent bays and elsewhere where high concentrations of dinoflagellates are present, the paths of swimming fish are clearly outlined by the bioluminescence they stimulate. Dolphins riding the bow wake of a ship are also cloaked in living light. Computer simulations of the bioluminescent wake of an aircraft carrier estimate it to be several km long, consistent with anecdotal reports of World War II pilots using the wake to locate their ship at night. Bioluminescence flow visualization can show complicated flow patterns, highlighting regions of flow separation and recirculation. Visualizing a turbulent jet with luminescent dinoflagellates reveals both the variability of instantaneous flow and the repeatable pattern of the average flow.

One engineering application of bioluminescence flow visualization is to highlight regions of high shear stress in bioreactors—culture vessels used to grow plant and animal cells *in vitro*. Although mixing in bioreactors is necessary for optimum mass



TOWDEX, the towed version of the HIDEX bathyphotometer, along with those involved in its development, including Prof. James Case of the University of California Santa Barbara (far right), a leader in the study of ocean bioluminescence.

[Images of plankton-stimulated bioluminescence associated with the swimming of fish (left), dolphin (middle) and swimmer (right).]



transfer of gases and nutrients, high shear stresses imparted by the mixing is detrimental to cells. Bioluminescence flow visualization allows the tradeoff between mixing and high shear stress to be evaluated for different bioreactor designs.

Once researchers understand the hydrodynamic basis of bioluminescence flow stimulation, they could use flow visualization as a quantitative tool to characterize complex flow fields that are not amenable to conventional approaches of studying fluid mechanics. One such endeavor is to determine levels of shear stress within a surface breaking wave. Although breaking waves are important in gas exchange, bubble formation and other important phenomena at the ocean surface, the physical processes within these waves are poorly understood.

A bright future

Luminescent organisms such as dinoflagellates possess unique anatomical, biochemical, molecular and behavioral specializations that demonstrate the important role of light emission in ecological processes. Our growing knowledge of dinoflagellate bioluminescence—especially of how flow stimulates light emission—provides an opportunity to use these microscopic organisms as calibrated flow markers in a variety of engineering and oceanographic contexts.

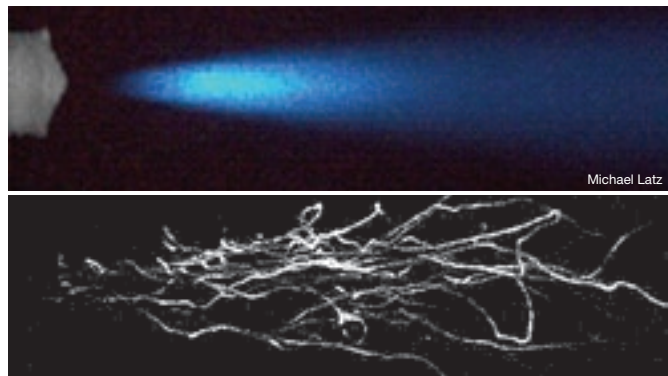
Advances in computational and experimental fluid mechanics offer new tools for investigation, while a new generation of digital light sensors expands researchers' ability to measure these dim light sources. To paraphrase physicist Ernst Mach, from studies of bioluminescence we can learn better physics.

Moreover, the natural beauty of this "living light" has intrigued and mesmerized researchers and non-scientists alike for ages. See you in Puerto Rico! ▲

Acknowledgments

Thanks to our collaborators for their contributions to this work, and to our sponsors, especially the Office of Naval Research, National Science Foundation and Space and Warfare Systems Center San Diego, for their financial support.

[Michael Latz (mlatz@ucsd.edu) is a marine biologist with the Scripps Institution of Oceanography, University of California, San Diego. Jim Rohr (james.rohr@navy.mil) is a physicist with the Space and Warfare Systems Center, San Diego.]



[Bioluminescence of the dinoflagellate *Pyrocystis fusiformis* stimulated by a turbulent jet created by flow through a 2 mm nozzle. (Top) A true-color 30 s time exposure image of the bioluminescence associated with the jet with an average velocity of 580 cm/s. The jet nozzle is seen at left. (Bottom) For the same configuration at a higher flow velocity of 1050 cm/s, a composite of multiple frames shows trajectories of individual flash events.]

[References and Resources]

Related Reading

- >> E.N. Harvey. *A History of Luminescence*, The American Philosophical Society, Philadelphia, 1957.
- >> K.J. Fleisher and J.F. Case. *Biol. Bull.* **189**, 263-71 (1995).
- >> J. Rohr et al. *J. Exp. Biol.* **201**, 1447-60 (1998).
- >> M.I. Latz and J. Rohr. *Limnol. Oceanogr.* **44**, 1423-35 (1999).
- >> M.I. Latz et al. *J. Exp. Biol.* **207**, 1941-51 (2004).
- >> M.I. Latz et al. *J. Plankton Res.* **26**, 1529-46 (2004).
- >> C.M. Herren et al. *Limnol. Oceanogr. Methods* **3**, 247-62 (2005).
- >> E.A. Widder et al. *Deep-Sea Res.* **40**, 607-27 (1993).
- >> J. Rohr et al. *Deep-Sea Res.* **49**, 2009-33 (2002).
- >> A.K. Chen et al. *Biotechnol. Bioeng.* **83**, 93-103 (2003).
- >> M.D. Stokes et al. *J. Geophys. Res.* **109**, C01004 (2004).
- >> Taylor, P. *Ocean Navigator* **80**: 90-8 (1997).
- >> Toner, M. *International Wildlife* **24**: 30-7 (1994).
- >> Trombley, J. *Pacific Discovery* **45**, 8-18 (1992).
- >> Widder, E.A. *Sea Technology March*: 33-9 (1997).

On the Web

The Latz Research Laboratory [<http://siobiolum.ucsd.edu>]
 Bioluminescence Web Page [www.lifesci.ucsb.edu/~biolum/]
 Harbor Branch Bioluminescence
 [www.hboi.edu/marinesci/biolum.html]
 Naval Oceanographic Office Bioluminescence Survey Systems
 [www.navo.navy.mil/biolum/blwebpge.htm]
 Oceanus Magazine
 [<http://oceanusmag.whoi.edu/v43n1/lippsett2.html>]