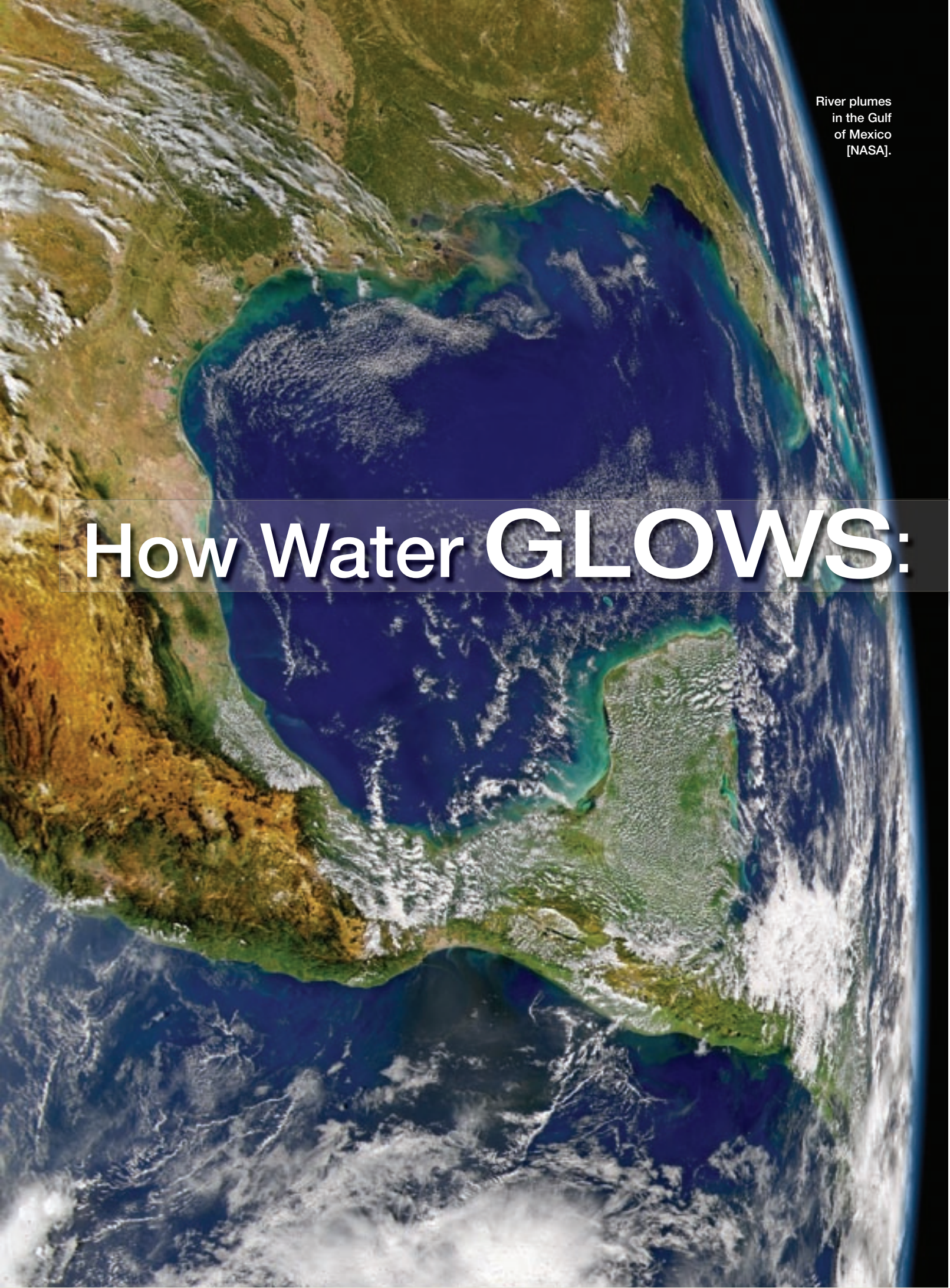
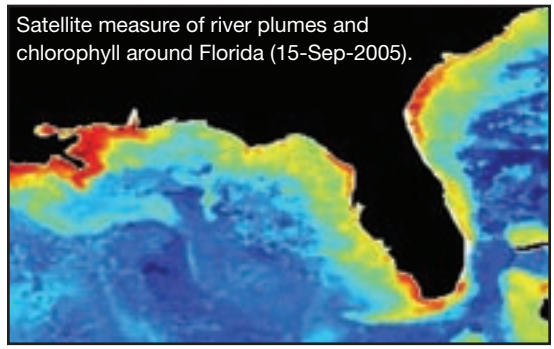


River plumes in the Gulf of Mexico [NASA].

How Water GLOWS:

Dennis Killinger
and Vasanthi Sivaprakasam



R. Weisberg and F. Muller-Karger, USF College of Marine Science

Water Monitoring with Laser Fluorescence

With an ever-increasing global need for fresh water, scientists must develop new instruments to allow for real-time, inexpensive detection and monitoring of water quality. Lasers and optical detection techniques offer a unique enabling technology that can be used toward this goal. The authors describe their deep UV (220 nm – 300 nm) laser-induced-fluorescence (LIF) system.

"Water, water everywhere,/ nor any drop to drink," wrote Samuel Taylor Coleridge in the 1798 poem *The Rime of the Ancient Mariner*, referring to the lack of potable water in the vast ocean. It is a statement as true today as it was 200 years ago. Although two-thirds of the Earth is covered in water, 97.5 percent is salt water. Among the remaining fresh water in the world, 69 percent is tied up in polar ice caps and glaciers, and at least 30 percent is in swamps or soil moisture. All told, less than 1 percent of fresh water is usable for humans—or about 0.02 percent of the total water on the planet.

According to a recent National Intelligence Council report titled "Global Trends: 2015," more than half of the world's population will live in countries that will be "water-stressed" by the year 2015, and one-half of the world's land surface will have river basins shared by more than one country. Given its critical role in health, agriculture and manufacturing, water has become a global commodity not unlike oil.

Most approaches for monitoring water quality in water processing plants are based on "wet" chemistry techniques that require the addition of other chemicals to water samples. Many use gas chromatography or liquid chromatography followed by laser fluorescence detection in the reagent capillary tube, while others use reagents that change pH, color or other physical characteristics depending on the concentration of selected or trace species. Although useful in the lab, these techniques work less well in the field, where reagents are difficult to replace and harsh conditions may degrade them.

To address these challenges, we have developed a reagentless, deep UV (220 nm – 300 nm) laser-induced-fluorescence (LIF) system for detecting certain contaminants and potentially

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Current Water Monitoring Systems and Future Trends

Water monitoring in the United States is guided by regulations and procedures developed by the U.S. Environmental Protection Agency (EPA). The EPA "Series 500" is a sequence of tests designed to identify and quantify organic compounds in municipal drinking water. One method in this series describes the detection of volatile organic compounds in water by purge and trap capillary column gas chromatography with photoionization and electrolytic conductivity detectors.

Such tests are conducted off-line and in the laboratory by state and local municipalities, which are responsible for maintaining adherence to EPA regulations or exceeding them. There are close to 160,000 water municipalities in the United States. Most municipal water processing plants test for water quality at the plant itself, using "wet" chemistry—or tests on water samples that are conducted with standard laboratory solvents. The techniques typically take anywhere from one hour to several days to complete.

Currently, no real-time or reagentless laser-induced-fluorescence systems have been authorized for use by water treatment plants. However, for the past several years, some water agencies have been testing a selected range of UV absorption and fluorescence water monitoring instruments. One such device is a UV-visible (200 nm – 750 nm) absorption instrument from S-CAN in Austria that can detect small changes in the optical absorption properties of water.

Another fluorescence-based test is used to monitor water for the bacteria *E. coli*. This involves growing a culture obtained from a water sample, using a fluorescence dye or stain, and counting the organisms, by either visual microscopes or laser readers. Fluorescence is also used in liquid chromatography laser-induced fluorescence, or LC-LIF, a technique in which a capillary tube is used to separate the chemical species and a laser reads the separated column.

>> For more information on the EPA's water regulations, visit **www.epa.gov/safewater/methods/methods.html**.

harmful substances in bottled and processed water and the ocean. The system has a sensitivity of about a few parts per trillion, which is several orders of magnitude better than conventional spectrofluorometers. It has detected the presence of plastic resins and dissolved organic compounds (DOCs) in bottled drinking water, monitored real-time changes in DOCs in reverse-osmosis-processed drinking water, and been used to track DOCs and river plumes in the Gulf of Mexico.

Previous LIF studies

We are not the first to have researched laser-induced fluorescence of water. Indeed, there have been many previous LIF studies of water, many of which have used either blue-green Argon ion lasers or doubled Nd:YAG lasers for excitation, as well as staining reagents for improving the contrast of detected organisms.

Studies that have relied on the natural or auto-fluorescence from water have generally not been as successful as those that have used fluorescence dyes. This is possibly because the blue-green laser wavelengths that were used in the former investigations did not sufficiently separate between the emission spectral peaks due to trace organics versus excitation or interfering background spectra.

As a result, few previous LIF studies have drawn on the natural fluorescence of trace species in the water. We attempted to overcome some of the limitations of the natural fluorescence approach by using a deep-UV laser source near 220 nm – 300 nm for excitation. The resultant fluorescence emission from the organics was well separated from the excitation wavelength.

New detection methods and UV laser sources

One of our major goals in this work has been to improve on the signal-to-noise ratio (S/N) of conventional spectrofluorometers by replacing the conventional CW or chopped xenon light sources with a high pulse-repetition-frequency (PRF) laser. We used high PRF-gated boxcar signal processing instead of the usual analog lock-in amplification. We also utilized the spatial coherence of the excitation laser beam to allow for multi-passing of the beam within the fluorescence cell.

By gating the boxcar processor only around the short (1 to 20 ns) laser and emitted fluorescence pulse, we were able to reject the detector noise in between the laser pulses. This technique is viable when the fluorescence lifetime of the emitting species such as the DOCs and plastic resins is short (10 to 20 ns). Assuming equal average laser or optical excitation intensity, the improvement, I, is on the order of the square root of the ratio of the laser pulse period and the fluorescence lifetime or boxcar gate time, or

$$I = (\tau_{laser} / \tau_{gate})^{1/2} \quad .$$

For example, in the case of a microchip laser operating at a PRF of 8.6 KHz (τ_{laser} of 0.116 ms) and a boxcar gate width of 50 ns, the improvement expected is close to 50.

The laser-induced-fluorescence system has a sensitivity of about a few parts per trillion, which is several orders of magnitude better than conventional spectrofluorometers.

Another way we were able to improve the S/N was by conducting high-speed pulse averaging of more than 8,600 pulses in one second; however, such an improvement applies equally to the CW or high-PRF pulsed laser case.

In our research, we used several LIF systems, which differed with regard to their laser tunability and spectroscopic detection techniques. We decided which to use based on whether we were planning to study a laboratory tunable spectral survey or deploy a fixed wavelength field LIF instrument.

For tunable excitation and laboratory spectroscopic studies, we used either (1) a large tripled Nd:YAG pumped optical parametric oscillator (doubled) to produce 10 to 30 mJ/pulse, 20 Hz PRF, tunable excitation from 220 nm to 350 nm; or (2) a smaller nitrogen laser pumped doubled dye laser with 1 μJ/pulse, 20 Hz and tunable from 220 nm to 320 nm.

We used the larger laser for initial spectroscopic work. However, because that laser photobleached many trace species unless its power was attenuated, we relied on the tunable doubled dye laser for most of the tunable spectroscopic survey work presented here.

Our field-deployable portable LIF unit included a fixed wavelength 266-nm microchip laser (1 μJ/pulse, PRF 8.6 kHz) and a 355-nm microchip laser that produced about a factor of 100 times the average power of the doubled dye laser. In all cases, we focused the output of the lasers into a 1-cm-wide quartz fluorescence cell, through which water samples flowed.

With a lens, we collected the emitted fluorescence, which was then analyzed with a spectrometer and cooled CCD detector or photo multiplier tube (PMT), or spectrally sorted by up to 21 optical bandpass filters and detected by a PMT. We compared the S/N of these configurations as well as that of a more moderate sensitivity/micro-spectrometer using quinine sulfate as a fluorescence standard.

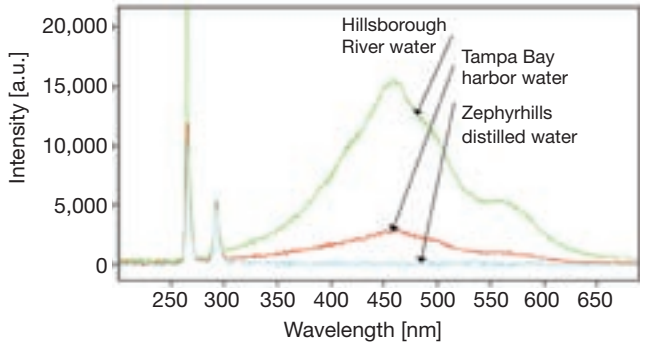
With the compact spectrometer as a baseline, the improvement was × 80 for the spectrometer CCD system and × 500 for the optical bandpass filter/PMT system.

Fluorescence from DOCs

We used the tunable laser system to measure the excitation emission matrix (EEM) spectra of DOCs in water. The analysis showed that excitation around 245 to 260 nm was near the absorption peak of the DOCs.

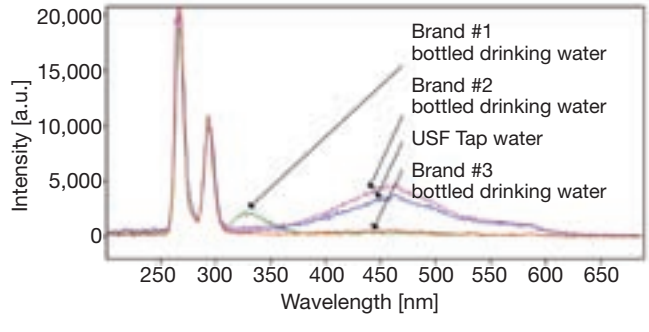
We used the 266-nm laser source and spectrometer/CCD detection system to obtain the fluorescence from various sources of natural water and a number of bottled drinking and distilled waters. In one analysis, we measured fluorescence from the local Hillsborough river, Tampa Bay harbor water and clean distilled water (Zephyrhills brand). In another, we measured tap water from the University of South Florida, along with three different brands of commercial bottled drinking water.

[Fluorescence emission from natural water samples]



Samples taken from a local river, Tampa Bay and clean distilled water using 266 nm laser excitation. The fluorescence near 450 nm is blue in color and is due to dissolved organic compounds (DOCs) in the water.

[Fluorescence emission from bottled water]



Three brands and tap water are assessed using 266 nm laser excitation. The fluorescence near 450 nm is due to dissolved organic compounds (DOCs) in the water while the small peak near 320 nm is due to leached plastic resins (bisphenol-A).

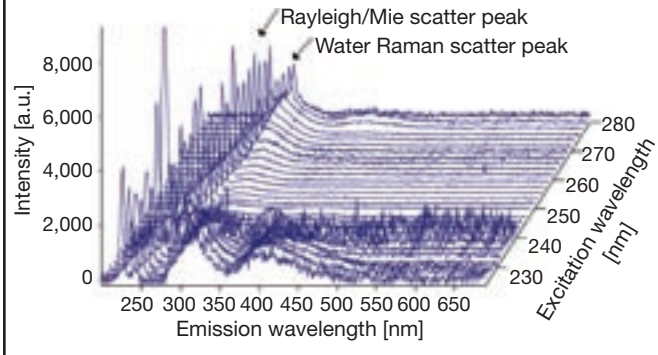
The emission spectrum from the natural water showed the attenuated Rayleigh-scattered light at 266 nm, a sharp peak near 290 nm from the Raman water scatter line and a peak near 450 nm from the DOCs in the water. The signal from the DOCs in the river water was very high because this river has a considerable amount of dissolved organics (tannins) from oak tree leaves.

The analysis of the bottled drinking water showed that two brands had almost no DOC levels, while another brand and the tap water had higher levels.

Fluorescence from plastic resins

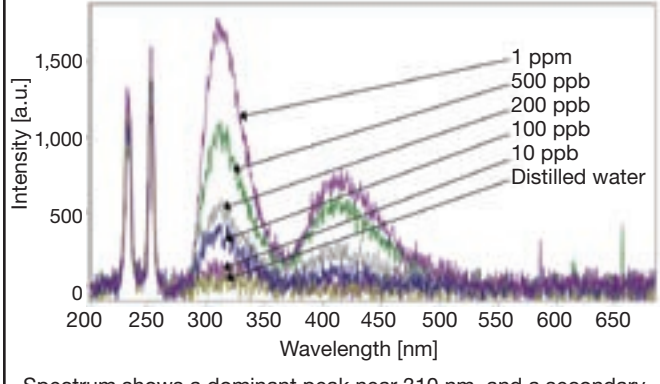
Our previous work in conjunction with Dr. Paula Coble (Marine Science/USF) had identified a plastic resin that was

Excitation-emission-matrix spectrum of bisphenol-A



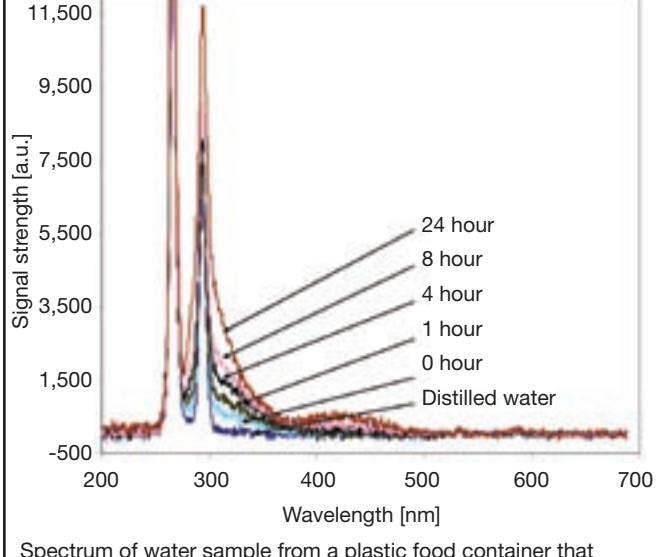
We assessed bisphenol-A, a plastic resin leached into water, using a tunable UV laser for excitation.

LIF spectrum from various concentrations of bisphenol-A



Spectrum shows a dominant peak near 310 nm, and a secondary peak near 420 nm from photo-decomposition products.

LIF emission for resins leached from plastic container



Spectrum of water sample from a plastic food container that was heated for three minutes in a microwave oven.

found to leach from underwater mines and plastic compounds into water. The resin was identified as bisphenol-A based on mass spectrometry studies. Human endocrine studies have also suggested that bisphenol-A leached from plastic containers and cans may be penetrating our environment and affecting human growth and development.

Along this line of study, we conducted extensive EEMspectral measurements by tuning the doubled dye laser from 220 nm to 340 nm. When the laser was tuned from 220 nm to 280 nm, the peak emission on an EEM spectra was near 330 nm, with a smaller peak near 400 nm at very short wavelengths and rebuilding at the longer wavelengths near 260 to 280 nm. However, the fluorescence seems to merge with the water Raman line at the longer wavelengths; this is why it is sometimes better to go to a very short wavelength that can be easily distinguished from that of a fluorescence line.

We conducted extensive LIF studies with bisphenol-A and established a detection sensitivity of a few hundred parts-per-trillion. On the measured spectrum for different concentrations of bisphenol-A, the primary peak near 310 nm is from bisphenol-A, while the secondary peak near 410 nm has been attributed to salicylic acid—one of the components that results from the photo-induced breakdown of bisphenol-A. In our experiments, we were careful in choosing the rubber and plastic tubing for our water flow system. We found that plastic leached out from a number of soft rubber or plastic tubing (silicone, C-Flex) and led to a fluorescence signature similar to bisphenol-A—near 310 nm. To minimize this problem, we switched to hard plastic—PTFE or PFA.

We observed similar plastic-related compounds leaching from everyday plastic containers, utensils and food storage bags. For example, we measured LIF spectra from distilled water placed in a microwavable plastic container, heated in a microwave oven for 3 minutes to 65° C, and then placed on a table in our lab. Although the water had cooled to room temperature within 15 minutes, the leached plastic concentration increased as a function of time over the next 24 hours.

Further studies showed similar results for water placed in plastic zip-lock bags at room temperature for a 24-hour period; this was accelerated if the samples were initially heated.

Portable LIF measurements in ocean water

The microchip lasers at 266 nm and 355 nm and the optical bandpass filter/PMT configuration produced the best overall LIF sensitivity and were configured to fit into two portable travel cases. This system was used for research aboard a USF research cruise vessel along with other wet chemistry and marine science instruments in order to correlate our real-time LIF data of DOCs and other spectral signatures.

We secured our LIF system to the cabin of the research vessel and connected sample lines into the ship's water flow that continuously pulled water from beneath the ship, into a de-bubbler, and out to the various work stations in the science cabin of the ship. We deployed hydrocast water sampling bottles,

The LIF sensor has potential to be used as a "trigger alarm" that could provide an early warning of any dangerous large-scale changes in DOC levels or other spectral signatures.

which were also lowered down into the ocean to bring up water samples for the other wet chemistry instruments. Our LIF system operated continuously, 24 hours per day, unattended.

We took a five-day research cruise that started in Key West, cruised through several river and effluent currents within the Gulf of Mexico, and ended in Tampa Bay. Plumes of DOCs were readily evident on the spectral analysis, with some coinciding with a plume of Red Tide detected by other on-board instruments. We also noted fluorescence near 680 nm that is due to the chlorophyll in phytoplankton. We plotted fluorescence over the five days at 450 nm (peak of DOCs), measured for the two excitation wavelengths at 266 nm and 355 nm and also that for the chlorophyll fluorescence near 680 nm. The two sets of data were well correlated, although there were deviations in some water masses.

Importantly, our LIF system still had a S/N level of about 100 in the clean, blue water that we encountered about 30 to 100 miles offshore. Due to the increased sensitivity of our LIF system compared to an on-board commercial spectrofluorometer, this was the first time that the USF Marine Science group had ever been able to measure fluorescence levels from the DOCs in clean water.

Looking ahead

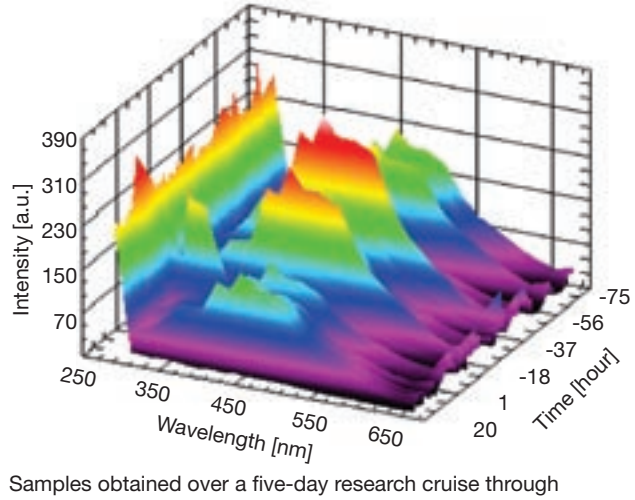
We have started to use our LIF system for monitoring impurities in water processed by a reverse osmosis (RO) unit. RO units have commercial and military uses, especially for the production of drinking water from salt or brackish water, and the purification of local ground water.

We have already used our LIF system for real-time detection and monitoring of the DOCs in the input/output stream of an RO unit, and are currently studying the EEM spectra to assess whether we could use one of the newly emerging compact UV diode lasers and LEDs in our LIF system. We are also studying the chemical makeup of the water and expect to be able to correlate our LIF spectral values with more conventional water instrument readings.

The LIF sensor has potential to be used as a "trigger alarm" that could provide an early warning of any potentially dangerous large-scale changes in the DOC levels or other spectral signatures. Other laboratory tests that use conventional water monitoring instruments could then be used to confirm the warning. This technique would be similar to some of the sensor systems currently being used by the Department of Defense to monitor chemical and biological agents. Along this line, we are exploring applications of our real-time reagentless LIF system to chemical and biological agent detection in water.

The authors gratefully acknowledge Dr. Paula Coble and Dr. Jennifer Boehme at USF/Marine Sciences for their extensive collaboration on the oceanic water studies, and Dr. Robert Carnahan and Miles Beamguard at USF/Engineering for help with the reverse osmosis research. We would like also like to thank Janet Jensen (Army EBCB), Marlyn Stasiak (City of Los Angeles Dept. of Water) and Yves Mikol (New York City EPA). Our work has been supported in part by the Office of Naval Research and the Army Research Office.

[LIF spectral data from Gulf of Mexico]



Samples obtained over a five-day research cruise through the Gulf of Mexico. The increased emission due to DOCs near 450 nm was correlated to outflow from river plumes into the Gulf.

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OSA Member

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