Next Generation of Advanced Laser Fluorescence Technology for Characterization of Natural Aquatic Environments

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LONG-TERM GOALS

The project research addresses our long-term goal to develop an analytical suite of the Advanced Laser Fluorescence (ALF) methods and instruments to improve our capacity for characterization of aquatic environments. The ALF technique (Chekalyuk and Hafez, 2008) sponsored in 2005-2008 by NOAA/CICEET and NSF uniquely combines high-resolution spectrally and temporally resolved measurements of the laser-stimulated emission (LSE). The real-time LSE spectral deconvolution provides assessments of key bio-geochemical variables, including chlorophyll-a (Chl-a), phycobiliprotein pigments, and chromophoric dissolved organic matter (CDOM). Three spectral types of phycoerythrin (PE) are discriminated for detection and characterization of PBP-containing phytoplankton and cyanobacteria. The spectrally-corrected pump-during-probe (PDP) measurements of variable fluorescence, F_v/F_m, yield improved assessments of phytoplankton photosynthetic capacity and biomass. An extensive series of flow-through ALF measurements in diverse water types, have demonstrated ALF utility as an informative integrated tool for aquatic research and observations. The ALF operational integration into the major oceanographic programs is currently in progress, including the California Current Ecosystem Long Term Ecological Research (CCE LTER, NSF) and California Cooperative Oceanic Fisheries Investigations (CalCOFI, NOAA).

OBJECTIVES

The specific goal of the project is to develop the next generation, commercial ALF sensors for oceanographic research, validation of satellite ocean color data, and environmental monitoring. The objectives are:

1. To develop the Aquatic Laser Fluorescence Analyzer (ALFA) for laboratory and field applications, including discrete sample analysis and underway shipboard measurements.
2. To develop the ALF In Situ (ALFIS) fiber-probe sensor.
3. To integrate, test and deploy the ALFA and ALFIS sensors on the solar-powered AUVs and research cruises.
4. To initiate operational use of the new ALF instruments for ecological and biogeochemical measurements, and validation of ocean color remote sensing.

The project research addresses NOPP BAA 2009 subtopics:

2.1 Integration of … in situ … bio-optical sensors on nontraditional or novel sampling platforms;
2.2A Development of the next generation of bio-optical field sensors to further exploit current "ocean color" satellite data, and/or new observations from ocean color satellite retrievals;
2.2B Development of enhanced or new laboratory instrumentation for ecological or biogeochemical measurements in support of ocean color remote sensing.

APPROACH

The approach is based on our broad understanding of the instrument development as a balanced combination of several core elements: the methodological research, instrument design, software development, calibration and validation, field tests and optimization, and integration of the research results with the synergistic interdisciplinary programs for their operational utilization. The project work is conducted in close collaboration between Lamont-Doherty Earth Observatory (LDEO) of Columbia University as a lead organization, WET Labs, Inc., as an industrial partner, and Scripps Institution of Oceanography (SIO). The basic suite of optical and electronic modules and software will be developed in Y1 and Y2 to integrate in the ALFA and ALFIS sensors (LDEO and WET Labs). The ALF analytical algorithms will be further refined via a series of laboratory and field measurements and integrated in the ALF software for real-time retrievals. The instrument design will allow sensor customization with regard to specific tasks and applications. The ALFA and ALFIS sensors will be calibrated and validated at LDEO, tested at SIO and operationally deployed on a series of research cruises in collaboration with the CCE LTER and CalCOFI programs. In addition, the new instruments will be integrated and field-tested on two solar-powered AUVs particularly suitable for validation of ocean color data and long-term autonomous deployments. The work plan includes analysis of the ALF field data for assessment of the accuracy thresholds and uncertainties in satellite retrievals of Chl-a, CDOM and other relevant variables. The project Work Plan includes the following tasks:

Task 1. Refinement of ALF analytical algorithms

Task 2. Development of Aquatic Laser Fluorescence Analyzer (ALFA)

Task 3. ALFA field tests and operational deployments

Task 4. Development of ALF In Situ sensor, ALFIS

Task 5. ALFIS field tests and operational deployments

Task 6. Validation of ocean color products using ALF field measurements

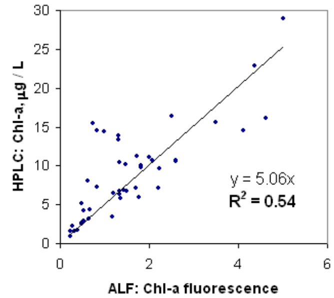
Key individuals: The **Principal Investigator**, Dr. **Alexander Chekalyuk**, is an expert in laser fluorescence, bio-environmental monitoring, and oceanographic research. Before joining Lamont-Doherty Earth Observatory (LDEO) of Columbia University, he worked as a research scientist at NASA GSFC, WHOI, and SIO. He has the relevant background in laser spectroscopy, fluorescence, bio-optics, technology, and phytoplankton photophysiology. He manages the project, leads the instrument development and modifications, plans and personally participates in the design and technological development, lab and field tests, and data interpretation. He communicates on a regular basis with the project team, and periodically visits the sub-awarded institutions to discuss the project work. Dr. **Andrew Barnard**, a project **CoI**, is a **WET Labs** Vice President of Research and Development. He manages all aspects of WET Labs work on engineering design and development of the ALFA and ALFIS instruments, including a redesign of all critical assemblies to improve mechanical and electro-optical stability and precision in coordination with the LDEO team. Mr. **Casey Moore** (WET Labs President) is involved in the project as WET Labs Principal Engineer; he also provides oversight on the programmatic activities. Dr. **James Sullivan**, Senior Research Scientist assists with the ALF analytical algorithms development, laboratory and field testing of the ALFA and ALFIS sensors. Dr. **Mati Kahru**, a project CoI from **Scripps Institution of Oceanography**, has a broad knowledge in biological oceanography and is a lead expert in ocean color satellite remote sensing. He serves as an interface between the project team and the SIO collaborators (Dr. Greg Mitchell, Prof. Mark Ohman, and Dr. Ralf Goericke), and plays a key role in developing and implementing the plan for assessment of the accuracy thresholds and uncertainties being used for both the remote sensing and sea-truth activities. He collects and analyzes the relevant data from the available satellite sensors in support of the ALF field deployments. Mr. **John Baker**, though not involved as a CoI, personally represents a project subcontractor, **Falmouth Scientific, Inc. (FSI)**, responsible for integration and deployment of the ALFIS sensor on the solar powered SUVII AUV developed by FSI. He is the President and General Manager of FSI. He has had 16+ years experience in the design and manufacture of digital and analog electronic systems. Dr. **Andrew Juhl**, Doherty Associate Research Scientist, is a biological oceanographer who assists this project by acquiring and maintaining phytoplankton cultures necessary for calibration of instrument prototypes during development and testing. He will also collect the water samples for instrument testing in the Hudson River Estuary.

WORK COMPLETED

Though the project start date was set by ONR as January 1, 2010, the project work was initiated in March 2010, after receiving the Y1 project funds by the lead organization, LDEO of Columbia University. Beginning of collaborative work between LDEO and the industrial partner was further delayed because the initially selected industrial partner, Turner Designs, has proposed a number of changes that would result in significant deviation from the original project plan, which might ultimately affect the project goals and objectives outlined in the approved proposal. LDEO has been able to consult with WET Labs that was willing to support the original project goals; the WET Labs proposal was fully consistent with the original scope of work as was their budget. The LDEO request to change the industrial partner was approved by ONR, and LDEO began the project work with WET Labs as a new industrial partner in mid May 2010. According to the Work Plan, the initial project work in Y1 was focused on the refinement of ALF analytical algorithms (Task 1), development of Aquatic Laser Fluorescence Analyzer (ALFA; Task 2), and validation of ocean color products using ALF field measurements (Task 6).

Task 1. *Refinement of ALF analytical algorithms*

As discussed in our proposal, normalization of chlorophyll-*a* (Chl-a) fluorescence to water Raman scattering results allows to improve the accuracy of fluorescence assessments of Chl-a concentration (Chekalyuk and Hafez, 2008). Nonetheless, the accuracy of underway Chl-a fluorescence retrievals is often compromised by photo-physiological regulation of Chl-a fluorescence yield. The concurrent ALF measurements of variable fluorescence, F_v/F_m, provide information about the photo-physiological status of phytoplankton and can be used to dramatically improve the accuracy of in vivo and in situ assessment of pigment concentration. We have processed and analyzed significant amount of recent field measurements to evaluate the effect of the Raman normalization and photo-physiological correction on the Chl-a fluorescence measurements. An example of improved correlation between the ALF measurements of Chl-a fluorescence normalized to water Raman and Fv/Fm and HPLC pigments retrievals is presented in lower panel of Fig.1 (R^2=0.92 vs. 0.54 in the upper panel representing non-corrected for Fv/Fm data of April-May 2008 from the Delaware and Chesapeake Bay). We plan to continue refining this approach to operationally include it in the ALFA and ALFIS measurements. Our effort was also focused on the ALF potential for detection and assessment of the phycobiliprotein (PBP)-containing photosynthesizing organisms in the mixed phototrophic populations. The ALF field measurements have been analyzed via comparisons of ALF retrievals of the group-specific PE spectral indexes with independent HPLC measurements of alloxanthin and zeaxanthin, the carotenoid biomarkers for the cryptophytes and cyanobacteria, respectively. On the other hand, our analytical work on fluorescence assessments of the algal photo-physiological status and chromophoric dissolved organic matter has not been completed as planned due to the delayed start of the project work (see above) and the time overlap with the emergent NSF-sponsored ALF field survey of the Oil Spill in the Gulf of Mexico (Aug-Sept 2010). We plan to catch up on these issues if our enclosed request for Y1 no-cost extension is granted by the ONR and other agencies participating in the NOPP program.



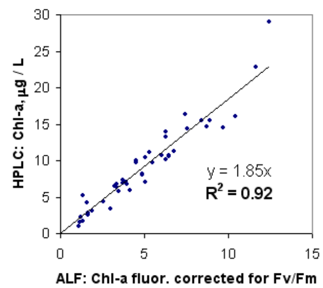


Figure 1. Correction of underway Chl-a fluorescence retrievals for photophysiological variability (F_v/F_m) significantly improves the accuracy of the underway and in situ fluorescence measurements of Chl-a concentration (R^2 = 0.92 vs. 0.54 for corrected and non-corrected data, respectively)

Task 2 *Development of Aquatic Laser Fluorescence Analyzer (ALFA)*
The ALFA instrument development is being conducted in partnership between LDEO and WET Labs. The ALF system design, component selection and measurement protocols has been critically analyzed to reduce the cost, size, weight, power consumption of the ALFA instrument, and to improve the overall system performance and robustness. The LDEO and WET Labs teams have held several project meetings to evaluate the overall architecture of the ALF prototypes and discuss the design options for the ALFA instrument (Fig. 2). The ALF components have been reviewed for potential improvements in reliability, manufacturability, and supportability. Bill of materials and functional specifications for the ALFA prototype has been produced. A redesign of all critical assemblies to improve mechanical and electro-optical stability and precision has begun, and we have transitioned the component and functionality specifications for the existing ALF units.

Figure 2. A project meeting at WET Labs to evaluate the ALFA design solutions.

Currently we are evaluating improvements to the design for rapid and cost effective commercial manufacturing. We have ordered the key components (the lasers, spectrometers, optics and mounting hardware) and initiated the breadboard investigations and system prototyping. A miniature low-power 16 mw 405 nm laser is being breadboard-tested to substitute the 50 mW laser used in the ALF prototype. Two new types of compact red-enhanced Hamamatsu photomultiplier modules will be evaluated in the current benchtop and future in situ instrument configurations. A new spectrometer with thermoelectrically cooled CCD sensor is being tested to substitute the older model used in earlier ALF prototypes. The optical design of the prototype ALF system is being modified to make it more robust and allow easy expansion and customization of the basic core ALFA configuration. A new lightweight modular rail optical mounting system has been successfully tested. The basic core ALFA configuration, $ALFA_V$, includes the 405 nm laser, CCD spectrometer, and PDP sensor of variable fluorescence to provide measurements of Chl-a, CDOM, and F_v/F_m. The upgrade to $ALFA_{VG}$ includes an additional 532 nm laser to provide spectral measurements of PBP fluorescence for characterization of PBP-containing cyanobacteria and cryptophytes. An external rugged PC laptop computer has been selected for the instrument control and data acquisition via a single water-proof USB2 port. We are currently reviewing the sampling protocols and existing software design. The prototype LabView software has been significantly reworked and modified to allow the instrument use by researchers and technicians without special training. The modified software was tested by our collaborator Dr. Goes (Bigelow) in July 2010 during the ALF deployment in the Amazon plume (Fig. 3) and, after further refinement, during the PI's field measurements of the environmental impact of the Deep Horizon Oil Spill in the Gulf of Mexico (Aug-Sept 2010).

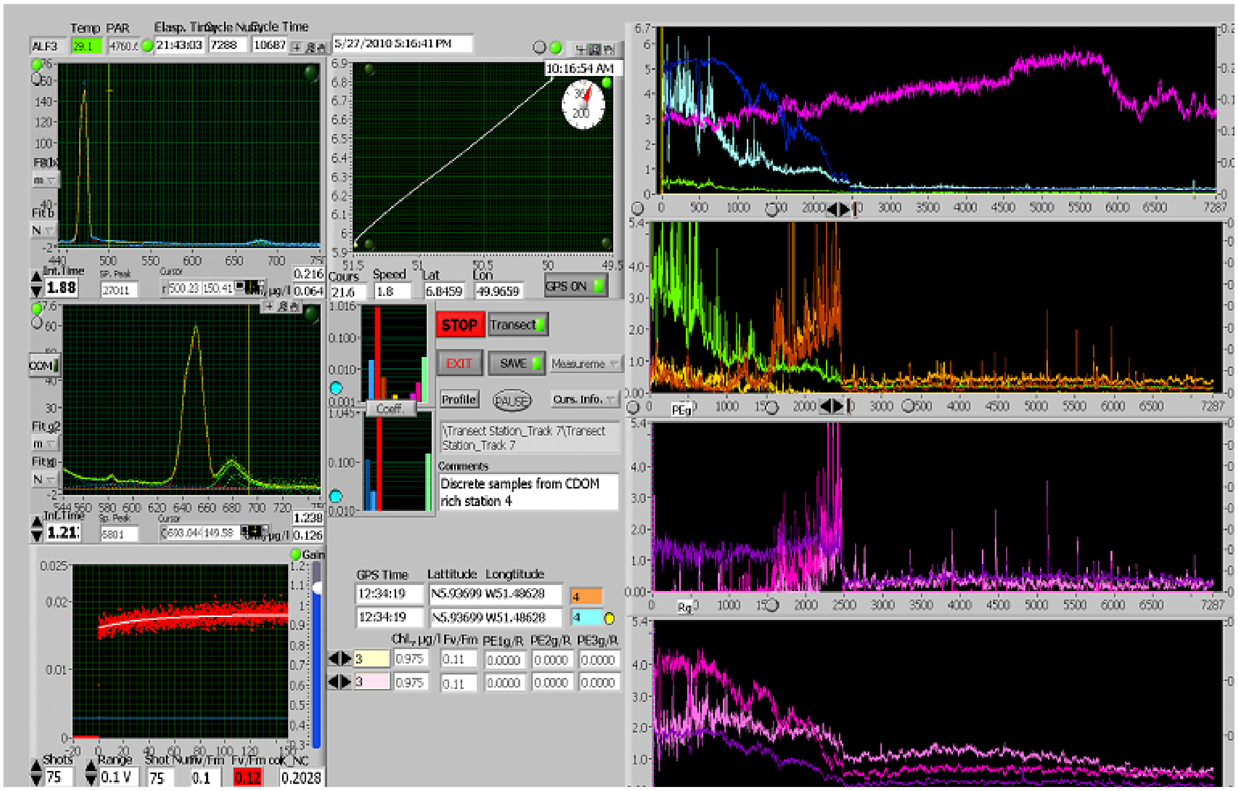


Figure 3. A screenshot of the simplified user interface of the new ALF operational software taken during ALF deployment in the Amazon plume, May 2010

The mechanical design is focused on the robustness and manufacturability of the instrument. The packaging is being designed in conjunction with the electronics and optics to make a compact waterproof enclosure that will allow for ease of assembly, repair, and use in the field. Currently, we are constructing two ALFA prototype units, with a completion date of December of 2010. The prototypes will be characterized at LDEO against existing ALF systems. Modifications to the design based on laboratory tests will be accomplished in this phase if necessary. Extensive field testing to verify performance will also be conducted in collaboration with SIO during winter and spring 2011 CalCOFI cruises and the CCE LTER Phase II Process cruise to refine the system and software design and finalizing the system specifications.

Task 6. *Validation of ocean color products using ALF field measurements*
The LDEO and SIO project teams have initiated their work on the comparative analysis of the existing ALF underway data vs. satellite retrievals. The ALF horizontal transect measurements has been shown to be useful for assessing the accuracy and uncertainties in satellite ocean color retrievals. ALF technique is uniquely positioned vs. 'regular' fluorometry as it yields more accurate assessment of pigment concentration and better addresses the different footprints of the satellite and typical in situ sensors. We have started working on the methodology of inter-comparisons between the ALF and satellite data sets to produce the quantitative statistical estimates. Our major effort was focused on the Chl-a concentration, the most important product of ocean color satellite measurements. Two data sets acquired in the contrast areas of California Current (October 2008, ALF team on the CCE LTER cruise) and Bering Sea (July 2008, Dr. Goes on the Healy cruise HLY0803, NSF) has been analyzed.

Due to high cloudiness, a significant challenge was to find the satellite images of reasonably good coverage in the Bering Sea. An example of the ALF validation of the satellite retrievals is presented in Fig. 4.

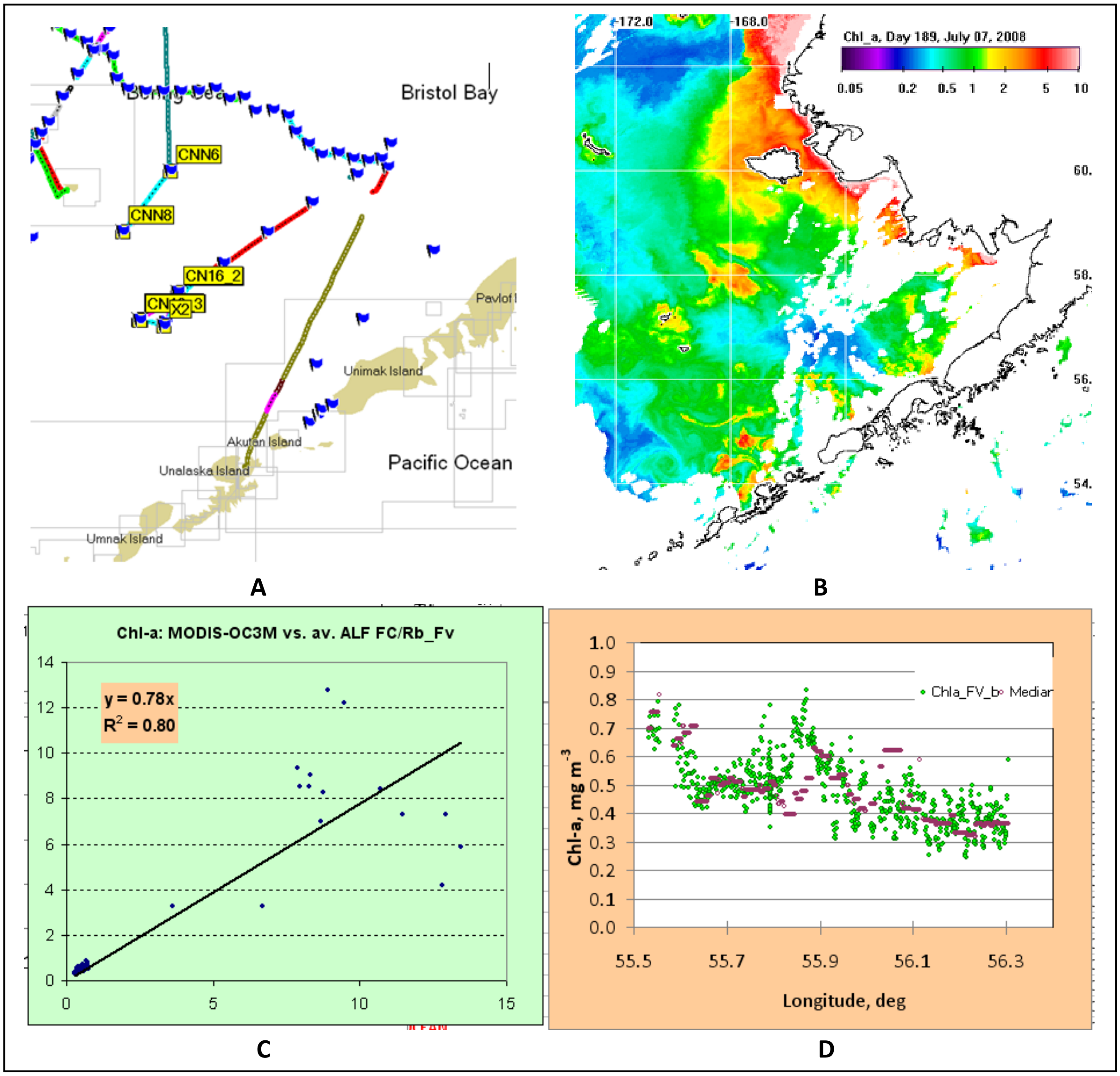


Figure 4. An example of the ALF valuation of satellite retrievals of Chl-a concentration.

The greenish lower diagonal line in panel A represents the transect line of ALF underway shipboard measurements. The MODIS Aqua Chl-a map (OC3M algorithm) is displayed in panel B. The HPLC Chl-a measurements of water samples has been used to calibrate the ALF fluorescence retrievals. As evident from panel D, the satellite retrievals (dark red) were in reasonably good agreement with the ALF Chl-a data (green) over most of the transect except its middle portion. The respective correlation plot displayed in panel C yielded $R^2 = 0.8$. As outlined in the proposal, after refining the approach with the existing ALF data, the new validation technique will be applied to the new measurements conducted during the field deployments of the ALFA and ALFIS sensors.

RESULTS

See the decription and examples of the meaningful results in the above section of the report.

IMPACT/APPLICATIONS

The project resaerch addresses NOPP BAA subtopics: 2.1 Integration of in situ bio-optical sensors on nontraditional or novel sampling platforms; 2.2A Development of the next generation of bio-optical field sensors to further exploit current "ocean color" satellite data, and/or new observations from ocean color satellite retrievals; 2.2B Development of enhanced or new laboratory instrumentation for ecological or biogeochemical measurements in support of ocean color remote sensing. The project builds upon state-of-the-art scientific and technological advances to provide the scientific community and government agencies new means for research, observations and environmental monitoring in diverse aquatic environments. The ALFA sensor will provide high-resolution shipboard underway flow-through measurements and sample analyses over a range of spatial and temporal scales. The ALFIS sensor will be used for deployments from a variety of platforms, including autonomous unmanned vehicles, automatic gliders, vertical and drift profilers, buoys, and moorings, thus contributing to development of the Ocean Observing Systems and other emerging initiatives.

RELATED PROJECTS

“Collaborative Research: Advanced Laser Fluorometer (ALF) for in vivo Characterization of Phytoplankton Pigments, Physiology and Community Structure”. NSF Ocean Technology and Interdisciplinary Research Program, Award # OCE-07-24561, May 2007- April 2010. Budget 462K, CoI: B. G. Mitchell (Scripps Institute of Oceanography, UCSD); Status: finishing project work under 1 year no-cost extension.

“RAPID: Rapid Assessment of Extent and Photophysiological Effects of the Deepwater Horizon Oil Spill”; NSF OCE; Award # OCE-1048482; June 2010 – July 2011; Budget: $199,972; CoIs: A. Subramaniam and A. Thurnherr (LDEO of Columbia University, NY); Status: in progress

REFERENCES

Chekalyuk, A.M. and M. Hafez. Advanced laser fluorometry of natural aquatic environments. Limnol. Oceanogr. Methods 2008, 6:591-609

PATENTS

“Analysis of Fluorescent Aquatic Constituents Using Spectral Deconvolution and Induction of Laser-Stimulated Emission” – A provisional patent application filed by the Science and Technology Ventures office of Columbia University in Nov. 2009. The patent application is in preparation to be submitted in Nov. 2010.