

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

Alexander Chekalyuk  
Lamont-Doherty Earth Observatory of Columbia University  
61 Route 9W, Palisades, NY 10964-8000  
phone: (845) 365-8552 fax: (845) 365-8150  
email: chekaluk@ldeo.columbia.edu

Andrew Barnard  
WET Labs, Inc  
620 Applegate Street, P.O. Box 518  
Philomath, OR 97370  
phone: 541 929 5650 fax: 541 929 5277  
email: andrew@wetlabs.com

Mati Kahru  
Integrative Oceanography Division  
Scripps Institution of Oceanography  
8622 Discovery Way, La Jolla, CA 92093-0210  
phone: (858) 534-8947 fax: (858) 822-0562  
email: mkahru@ucsd.edu

Award Number: N000141010205

<http://www.nopp.org/funded-projects/fy2009-projects-funded-under-nopp>

### **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) uniquely combines spectrally and temporally resolved measurements of the laser-stimulated emission (LSE) to provide assessments of key variables, including chlorophyll *a* (Chl *a*), chromophoric dissolved organic matter (CDOM), and phycobiliprotein-containing phytoplankton and cyanobacteria. The pump-during-probe measurements of variable fluorescence,  $F_v/F_m$ , yield assessments of phytoplankton photophysiological status. An extensive series of ALF measurements in diverse water types has demonstrated ALF utility as an integrated tool for aquatic research and observations. The ALF 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 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 was selected, designed and developed in Y1 and Y2 to integrate in the ALF sensors. The ALF analytical algorithms were refined via a series of laboratory and field measurements and integrated in the ALF software. The ALF sensors have been 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. 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. Key individuals: The Principal Investigator, Dr. Alexander Chekalyuk, is an expert in laser fluorescence, bio-environmental monitoring, and oceanographic research. Dr. Andrew Barnard, a project CoI, is a WET Labs Vice President of Research and Development. Mr. Casey Moore (WET Labs President) is involved in the project as WET Labs Principal Engineer. Dr. Mati Kahru, a project CoI from Scripps Institution of Oceanography (SIO) is a lead expert in ocean color satellite remote sensing. Dr. Andrew Juhl, Doherty Associate Research Scientist, is a biological oceanographer who assists this project by maintaining phytoplankton cultures necessary for calibration of the instruments. Mr. Mark Hafez, Research Associate, LDEO of Columbia University, ALF software, measurements, and data analysis.

## **WORK COMPLETED**

The **Y3 project work** was conducted in a series of Tasks outlined in the Work Plan of the original proposal:

- 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 (ALFIS) sensor

A new type of green laser emitting at 514 nm was successfully tested as a source of fluorescence excitation for both ALFA and ALFIS instrument configurations. The ALF analytical algorithms were further refined and updated to provide assessments of the key aquatic fluorescence constituents and phytoplankton photo-physiological parameters with the new 514 nm laser excitation. Various ALFA instrument configurations were developed on this basis. The dual-laser ALFA instruments built in Y1-Y2 were upgraded to expand the ALF analytical capabilities. A new optical "T" scheme was designed and implemented in two new ALFA prototypes: (i) a compact single-laser ALFA-T-514 instrument

that provides a basic set of measurements comparable to the earlier developed dual-laser instruments, and (ii) an advanced three-laser instrument for measuring an extended set of parameters. The latter instrument was equipped with 375, 405, and 514 nm lasers and deployed in May-July and Sept. 2012 in the Gulf of Mexico to assess consequences and bio-environmental impacts of the Deepwater Horizon oil spill of 2010. The various versions of the ALFA instrument were extensively tested in a series of field deployments in the Bering Sea (May 2012), Gulf of Mexico (Apr., May-July, and Sept. 2012), Amazon River plume (July 2012), and California Current (July-Aug. 2012). The breadboard prototype of the ALFIS instrument was developed on the basis of the 514 nm laser, fiber-probe, and dichroic beamsplitter. The ALFIS design solutions and instrument components were laboratory tested and refined. The project activities has resulted in two peer-review publications and two provisional patent applications. A brief description of the Y3 project results and accomplishments can be found below in the “Results” section.

## RESULTS

**Task 1. Refinement of ALF analytical algorithms.** The ALF analytical algorithms were further refined and updated. A new set of fluorescence components was developed and tested in the field for spectral deconvolution (SDC) of the laser-stimulated emission (LSE) measured with a new type of 514 nm laser to be used in both ALFA and ALFIS instruments (for example, see Fig. 2a, and Fig. 8 (middle-right panel in the screen capture). Accounting for photo-physiological variability of in vivo chlorophyll fluorescence (CF) per unit of chlorophyll concentration (CC) was incorporated in the analytical protocols to improve the accuracy of CC assessments. ALF field measurements of CF and photosystem II (PSII) photochemical yield ( $F_v/F_m$ ) were analyzed vs. independent CC retrievals in diverse marine environments (for example, Fig. 7). A four-step measurement protocol (Chekalyuk and Hafez, 2011) was successfully tested in the Gulf of Mexico and at other locations. It provides the accuracy of CC fluorescence measurements comparable to the accuracy of commonly accepted preparatory methods (for example, Figs. 7.1. and 7.3). For in situ or flow-through measurements, concurrent  $F_v/F_m$  measurements can be used to adjust for CF non-photochemical quenching (NPQ) and improve the accuracy of CC fluorescence assessments (Chekalyuk and Hafez, 2011). Our Y3 field tests have confirmed the NPQ-invariance of  $CF/(F_v/F_m)$  parameters and their high correlation with independently-derived CC magnitudes (Figs. 7.2 and 7.4). This method was used to convert the high-resolution underway ALF CF measurements of into the CC distributions during our Y3 deployments in diverse marine environments (for example, see Fig. 6 A,B). The ALF spectral deconvolution (SDC) algorithms were further updated to analyze the emission spectra stimulated with UV 375 nm excitation used in the Gulf of Mexico for surveying the bio-environmental impact of the 2010 Deepwater Horizon oil spill (see below).

**Task 2. Development of Aquatic Laser Fluorescence Analyzer (ALFA).** We continued our work on development of the benchtop ALFA instrument. The basic ALFA configuration, which we developed in Y1-Y2, was based on the X-scheme optical design incorporating 405 and 532 nm lasers. In Y3, a new type of green laser emitting at 514 nm was successfully tested as a source of fluorescence excitation to conduct both spectrally and temporally resolved ALF measurements that required the dual-laser excitation in the earlier design. A new optical design (“T” scheme) was developed by the LDEO team to incorporate the 514 nm laser in the ALF instrument (upper photo in Fig. 1). It provides easy access to the newly designed swappable sample compartments for flow-through measurements and discrete sample analysis. The easy access allows use of disposable plastic cells for the discrete sample analysis, which simplifies the instrument service and maintenance and reduces the sample volume needed for analysis. A compact ALF-T-514 instrument was built by the LDEO team in Jan.

2012 using the 514 nm laser (upper photo in Fig. 1). This development has resulted in the provisional patent application (# 2413.138prv; see below). The ALF-T-514 instrument was later upgraded to a three-laser configuration ALF-T-375/405/514 and operationally deployed in the Gulf of Mexico (May-July and Sept. 2012) and California Current (July-Aug. 2012). The UV laser emitting at 375 nm was used in conjunction with the 405 nm laser for fluorescence detection of oil and oil products (see above). The use of 375 and 405 nm lasers also helped to improve sensitivity of the CDOM measurements in offshore blue oceanic waters (e.g., Figs. 8, 9). Two dual-laser ALFA instruments built in Y1-Y2 were also upgraded with the 514 nm laser to provide an alternate 405/514 nm laser fluorescence excitation (ALFA-X-405/514), thus expanding the ALF analytical capabilities. The upgraded ALFA-X-405/514 instrument was successfully demonstrated at the AGU/ASLO Ocean Sciences meeting (Salt Lake City, Feb. 2012; see lower photo in Fig. 1), extensively tested in the field (Bering Sea (May 2012), Gulf of Mexico (Apr., May-July 2012), and Amazon River plume (July 2012)), and selected as a prototype for commercial version of the ALF instrument.

**Task 3. ALFA field tests and operational deployments.** An extensive series of field deployments was conducted in Y3 to test the new instrument design and refine the measurement protocols and analytical algorithms (Figs. 3-9). The various ALFA instrument configurations were deployed in the Bering Sea (May 2012), Gulf of Mexico (Apr., May-July, and Sept. 2012), Amazon River plume (July 2012), and California Current (July-Aug. 2012). An example of field measurements with the ALFA-X-405/514 instrument in the Gulf of Mexico conducted in collaboration with Dr. Antonietta Quigg (Texas A&M University) is displayed in Figs. 5-7. Along with the accurate high resolution measurements of Chl concentration and CDOM (Fig. 6 A,B), the new 514 nm excitation source was successfully tested for fluorescence measurements of phycobiliprotein (PBP) pigments to provide basic structural characterization of the phytoplankton communities (Fig. 6 D,E). A new analytical capability for measuring variable fluorescence,  $F_v/F_m$ , using the alternate blue/green excitation has confirmed that various groups of phytoplankton can be in different physiological state under identical environmental conditions. As evident from Fig. 6C, the difference between  $F_v/F_m$  magnitudes measured with 405 (B) and 514 (G) nm excitation varied along the transect from 48% to 10%, reflecting the structural changes in the phytoplankton composition displayed in Fig. 6D,E. The largest difference was observed in the most offshore area in the middle of transect line (Fig. 5), where cyanobacteria were found dominant in the phytoplankton population (Fig. 6E). Since cyanobacteria are known to more efficiently absorb green than blue light, the ALF transect measurements indicates that the cyanobacterial sub-population was physiologically better adapted to the offshore environmental conditions than eukaryotic, not-containing PBP pigments phytoplankton.

In May-June 2012 both ALFA-X-405/514 and ALF-T-375/405/514 instruments were used on the ECOGIG cruise focused on the assessment of consequences and bio-environmental impacts of the 2010 Deepwater Horizon oil spill in the Gulf of Mexico. In Fig. 8, we show an example of the underway ALFA-X-405/514 measurements during the ECOGIG cruise along the transect line highlighted with yellow in the map (upper panel). Consistent with our earlier observations in the California Current (Chekalyuk et al., 2012), we observed strong phytoplankton responses (including sharp changes in pigment biomass, physiological state, and community composition (see two middle-right plots in lower panel of Fig. 8) when crossing the the frontal zone characterized by significant changes in physical and chemical properties (lower right panel in Fig. 8). In addition, a new  $F_v/F_m$  measurement protocol using the alternative 405/514 nm excitation showed strikingly different patterns across the frontal zone (upper right plot in Fig. 8). While  $F_v/F_m G$  magnitudes (cyan line; measured with the green laser more efficiently stimulating cyanobacterial fluorescence) showed gradual increase with decreasing salinity along the transect line, the  $F_v/F_m B$  values (magenta line; measured with the blue laser more efficiently stimulating fluorescence of eukaryotes) indicated a sharp frontal decline in

phytoplankton photophysiological status across the salinity gradient, where elevated concentration of green-water cyanobacteria was detected (orange peak in the upper-mid right plot in Fig. 8). This example clearly demonstrates the unique observational and analytical capabilities of the new dual-laser ALFA instrument configuration.

Along with testing the new instrument configurations, we kept deploying the earlier developed ALFA-X-405/532 instruments for side-by-side comparison with the new technology. An example of such measurements across a frontal zone in the California Current is displayed in Fig. 9. Similar to data shown in Fig. 8, strong changes in phytoplankton pigment biomass, physiological status, community composition, and CDOM concentration were detected by the ALF measurements. These measurements were part of the large-scale ecological survey conducted by the NSF CCE LTER program, with which we have long-term collaboration. In fact, we have already reached the goal formulated as objective 4 in the original proposal, and the ALF measurements have become an important part of the CCE LTER program.

**Task 4. Development of ALF In Situ (ALFIS) sensor.** Development of the In Situ version of the ALF instrument (ALFIS) is a technologically challenging task. It requires new design and technological solutions, as well as careful selection of the instrument components to develop a compact, robust, low power instrument capable of long-term autonomous operation in the chemically and biologically aggressive seawater under high pressure. The breadboard prototype of the ALFIS instrument was developed during Y3 work by the LDEO ALF project team, based on the 514 nm laser, fiber-probe, and dichroic beamsplitter. It incorporates several unique design solutions and components described in detail in our recent patent application (below, Docket No.: 2413.141PRV). The key design solutions are the use of the single-laser 514 nm excitation, the dual-leg fiber probe to deliver the excitation to the water volume and the laser-stimulated emission back to the instrument sensors, and the dichroic filter splitting the signal between the instrument sensors. An operational breadboard prototype of the fiber-probe ALFIS instrument is shown in Fig. 10. It includes a miniature spectrometer, 14-bit waveform digitizer, and small, low power PC computer. Fig. 11 shows an example of spectrally and temporally resolved test measurements that provide signal-to-noise ratio comparable to the benchtop ALFA instrument. The WET Labs and LDEO project teams are currently working on developing the submersible ALFIS prototype based on this breadboard ALFIS design to initiate its laboratory and field tests in a few months. After the initial tests at the SIO pier, the Moving Vehicle Profiler and the SeaSoar towed vehicles (Fig. 12) are considered as prospective platforms for deployments of the ALFIS instrument.

## **IMPACT and APPLICATIONS**

The project research 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.

## **Economic Development**

WET Labs is a company that works at the forefront of technology and provides state-of-the-art oceanographic sensors and platforms. The new ALFA and ALFIS sensors will create conceptually new line of products enabling new unique measurement and observational capabilities. The extensive series of field deployments of the prototype ALF instrument has already attracted significant interest to the new technology in the research community and government agencies. The completion of this NOPP project and commercialization of the ALF technology will create new jobs and promote local and regional economic development.

## **Quality of Life**

The ALFA and ALFIS sensors will provide measurements of the key bio-environmental variables, including chlorophyll-a biomass, phycobiliprotein pigments, chromophoric dissolved organic matter, as well as basic characterization of the structure and photo-physiological status of the mixed populations of phytoplankton and cyanobacteria in natural waters. The operational use of the new technology in oceanographic research and environmental monitoring will provide new observations and data to address the emerging problems of particular importance in coastal, estuarine and inland aquatic environments, such as ecosystem health and biodiversity, harmful algal blooms, eutrophication, pollution, fisheries, coastal management, etc., which may ultimately help to improve the quality of life.

## **Science Education and Communication**

The potential future impact of developing the next generation on the ALF technology will include introduction of the the new scientific data and developments incorporated in the ALF measurement and analytical algorithms to the engineering community and public sector. The initial results of our NOPP project have been reported at the XX Ocean Optics meeting (Anchorage, Alaska). Several talks and presentations on the ALF technology, applications and commercialization and are planned at the Special Session # 127 on Pigment Fluorescence aorganized by the Project PI, Dr. Chekalyuk, at the AGU Ocean Sciences meeting in Feb. 2012 at Salt Lake city (Utah). These meetings are attended by significant amount of undergraduate and graduate students and researchers. We will continue informing the scientific community and public about our NOPP project results and accomplishments via publications, presentations and internet web sites.

## **TRANSITIONS**

### **Quality of Life**

The California Cooperative Oceanic Fisheries Investigations (CalCOFI, NOAA) program has already deployed the prototype ALF instruments in collaboration with LDEO of Columbia University on several seasonal CalCOFI cruises in the Pacific coastal zone of California and California current to measure spatial patterns of the key bio-environmental variables over large aquatic areas. They will collaborate with our NOPP project team on the field tests and deployments on the new technology to ultimately incorporate it into the long-term CalCOFI monitoring program. The successful operational incorporation of the ALF technology into the CalCOFI program may provide new data to improve California coastal fisheries and resource management.

### **Science Education and Communication**

The California Current Ecosystem Long Term Ecological Research (CCE LTER, NSF) program has already deployed the prototype ALF instruments on several research cruises in the Pacific coastal zone of California and California current. The program has been recently extended by NSF for additional 6 years with specific emphasis on the role of meso-scale physical and biological structures on the California Current Ecosystem. They have already ordered a prototype of the ALFA system that they will use on their research cruises and during the laboratory studies. The instrument will be built in December 2010 and transferred to Scripps Institute of Oceanography, the lead organization of the CCE LTER program. Our NOPP project team will train the CCE LTER personnel and participate in the field deployments. The acquired data will be used to provide important feedbacks to further improve the new generation of the ALF technology.

## **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: completed in 2011.

“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: Completed in 2012.

## **REFERENCES**

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

A. Chekalyuk and M. Hafez, "Photo-physiological variability in phytoplankton chlorophyll fluorescence and assessment of chlorophyll concentration," *Optics Express*. **19**(23), 22643–22658 (2011)

Chekalyuk, A.M., M.R. Landry, R. Goericke, A.G. Taylor, and M. Hafez. Laser fluorescence analysis of phytoplankton across a frontal zone in the California Current ecosystem. *J. Plankton Res.*, 34(9), 761-777 (2012)

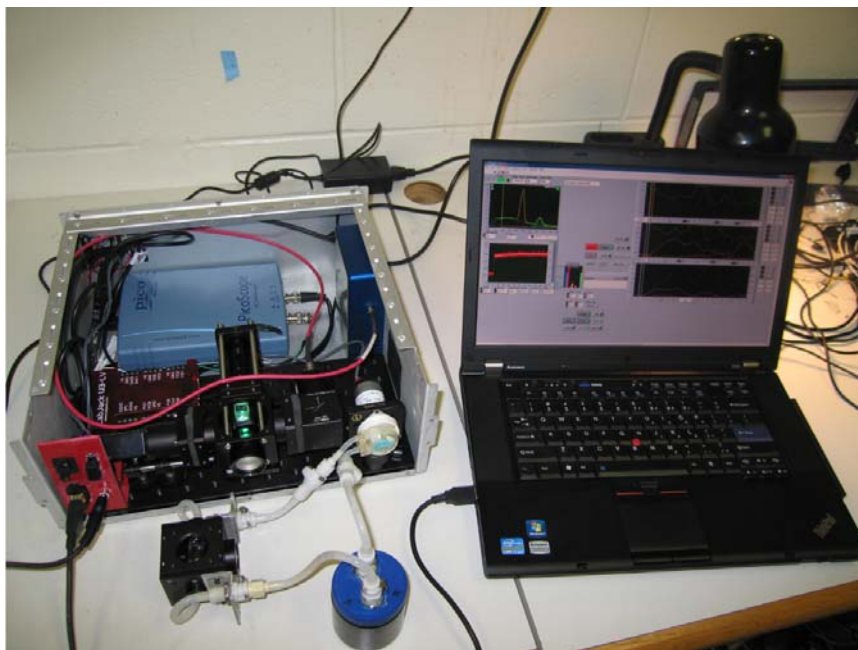
## **PUBLICATIONS**

Chekalyuk, A.M., M.R. Landry, R. Goericke, A.G. Taylor, and M. A. Hafez. Laser fluorescence analysis of phytoplankton across a frontal zone in the California Current ecosystem. *J. Plankton Res.*, 34(9), 761-777 (2012)

## **PATENTS**

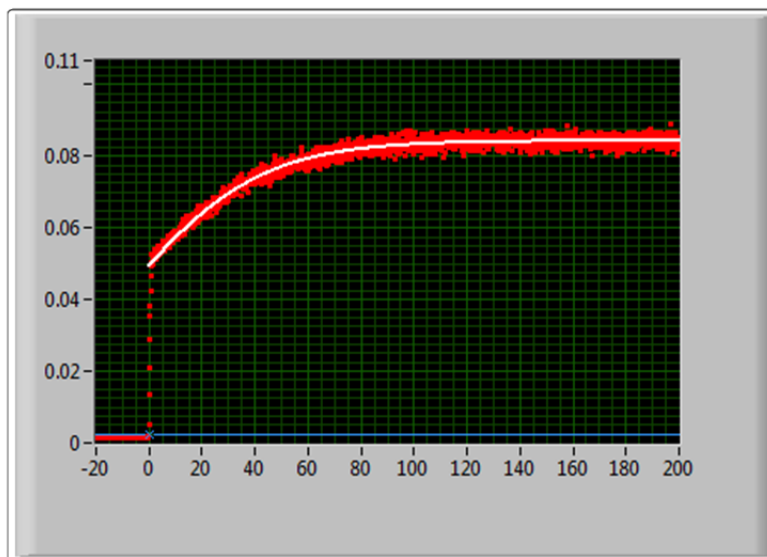
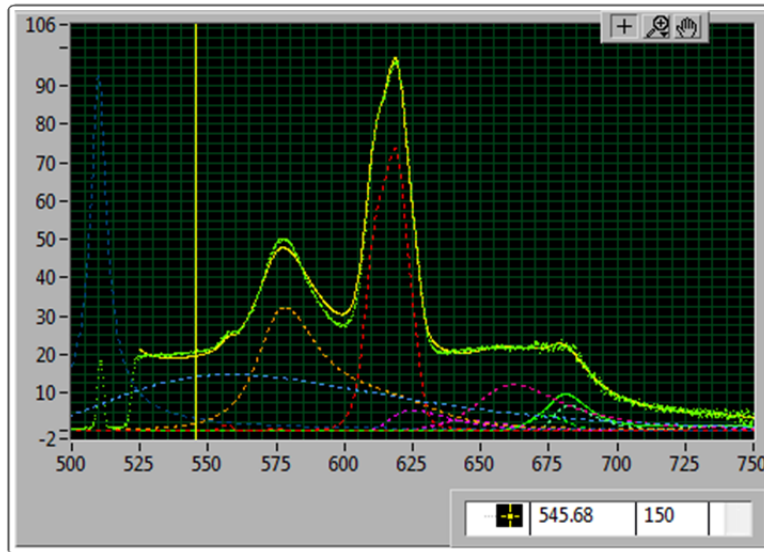
Provisional patent application: “Optimized Optical Setup for Stimulation, Collection and Spectral Filtration of Optical Emission in Liquids”. Inventor: Alexander Chekalyuk (filed with the Science and Technology Ventures of Columbia University on Feb 3, 2012; reg. # 42,267; Docket No.: 2413.138PRV).

Provisional patent application: “An Optical Setup for Spectrally and Temporally Resolved Measurements of Laser-Stimulated Emission in Natural Waters”. Inventor: Alexander Chekalyuk (filed with the Science and Technology Ventures of Columbia University on July 30, 2012; reg. # 42,267; Docket No.: 2413.141PRV)..





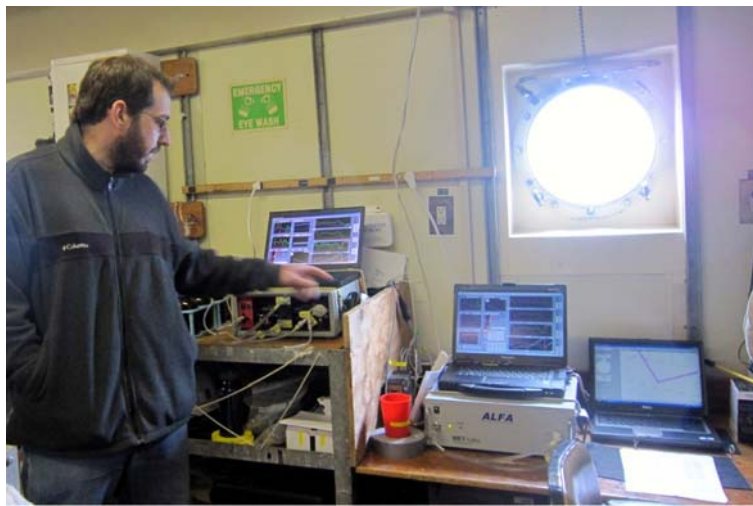
*Fig. 1. Upper: A new prototype of the benchtop ALF instrument that implements a new optical design (“T-scheme”) and incorporates a new type of 514 nm laser to provide both spectrally and temporally resolved fluorescence measurements. The new design provides easy access to the swappable sample compartments for discrete sample analysis and flow-through measurements, and upgradability for multi-laser excitation (provis. patent application # 2413.138prv of 02/03/12). Lower: The ALFA instrument upgraded with the 514 nm laser was demonstrated at the AGU/ASLO Ocean Sciences meeting (Salt Lake City, Feb. 2012).*



*Fig. 2. An example of spectrally (upper, emission wavelength in nm along X axis) and temporally (lower, time in  $\mu\text{s}$  along the X axis;  $F_v/F_m=0.44$ ) resolved ALF measurements of phytoplankton pigment fluorescence using the new optical design (see Fig. 1) and spectral correction for the back reflection. The new set of spectral components was developed for the spectral deconvolution (SDC) analysis of the laser-stimulated emission (LSE).*



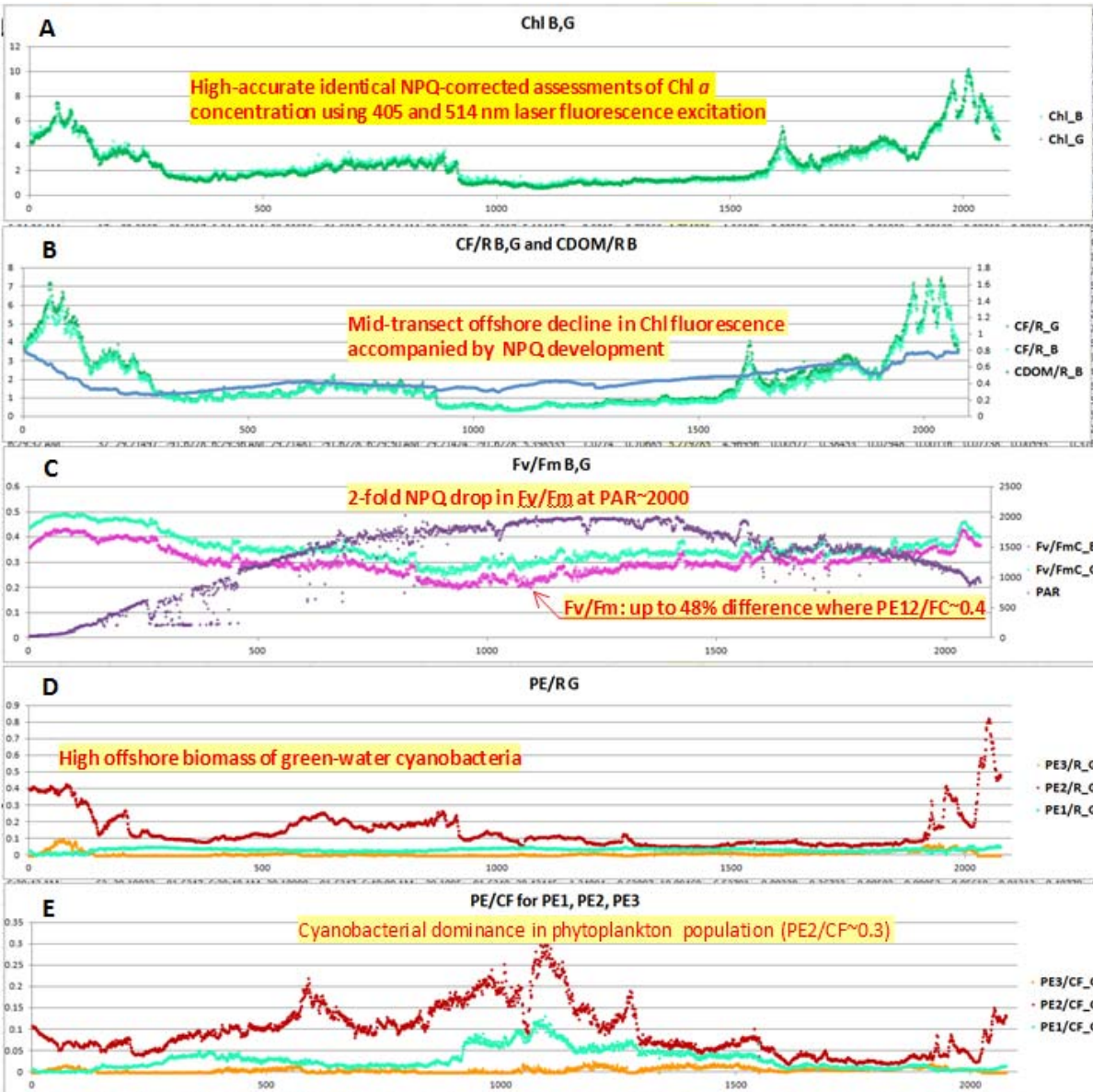
***Fig. 3. The new ALF-T instrument prototype (left) and the upgraded with 514 laser ALFA-X-405/514 instrument were extensively tested in a series of field deployments in the Bering Sea (May 2012), Gulf of Mexico (Apr., May-July, and Sept. 2012), Amazon River plume (July 2012), and California Current (July-Aug. 2012).***



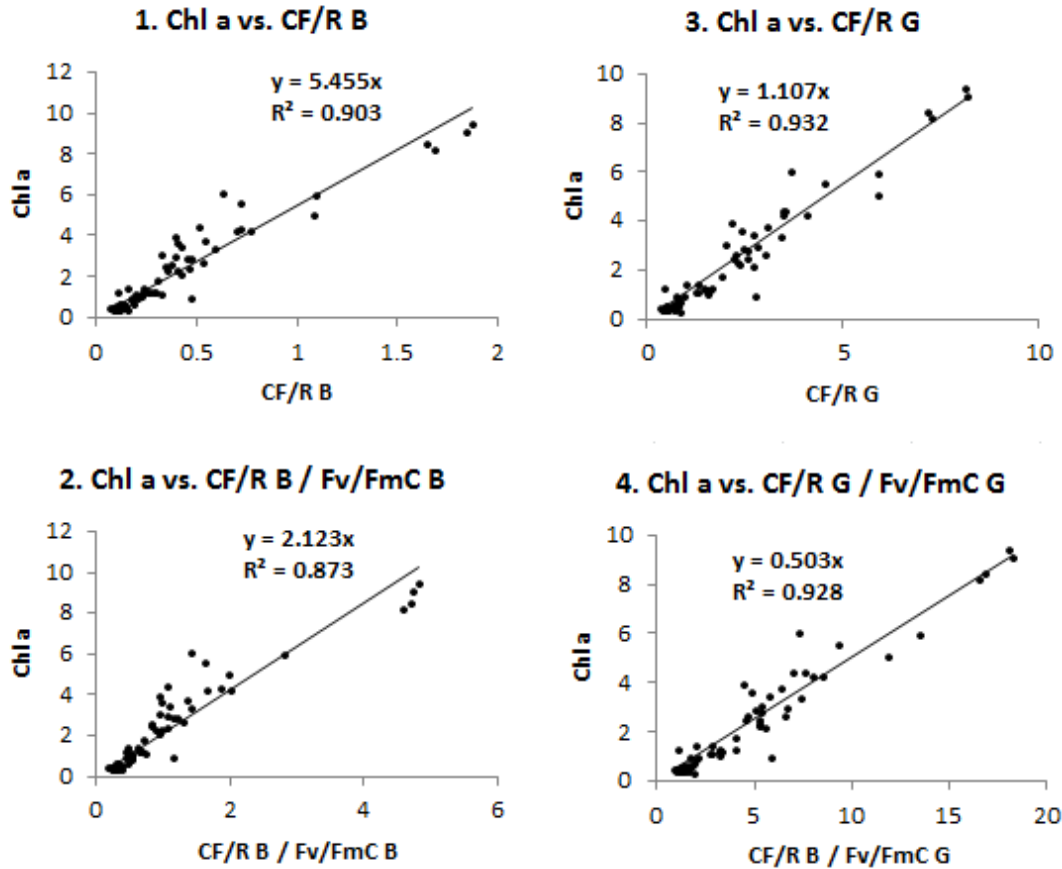
***Fig. 4. Field deployment and tests of the ALFA instruments by the LDEO project team in collaboration with Scripps Institution of Oceanography on the NSF-sponsored California Current Ecosystem Long Term Ecological Research (CCE LTER) cruise, June-July 2012.***



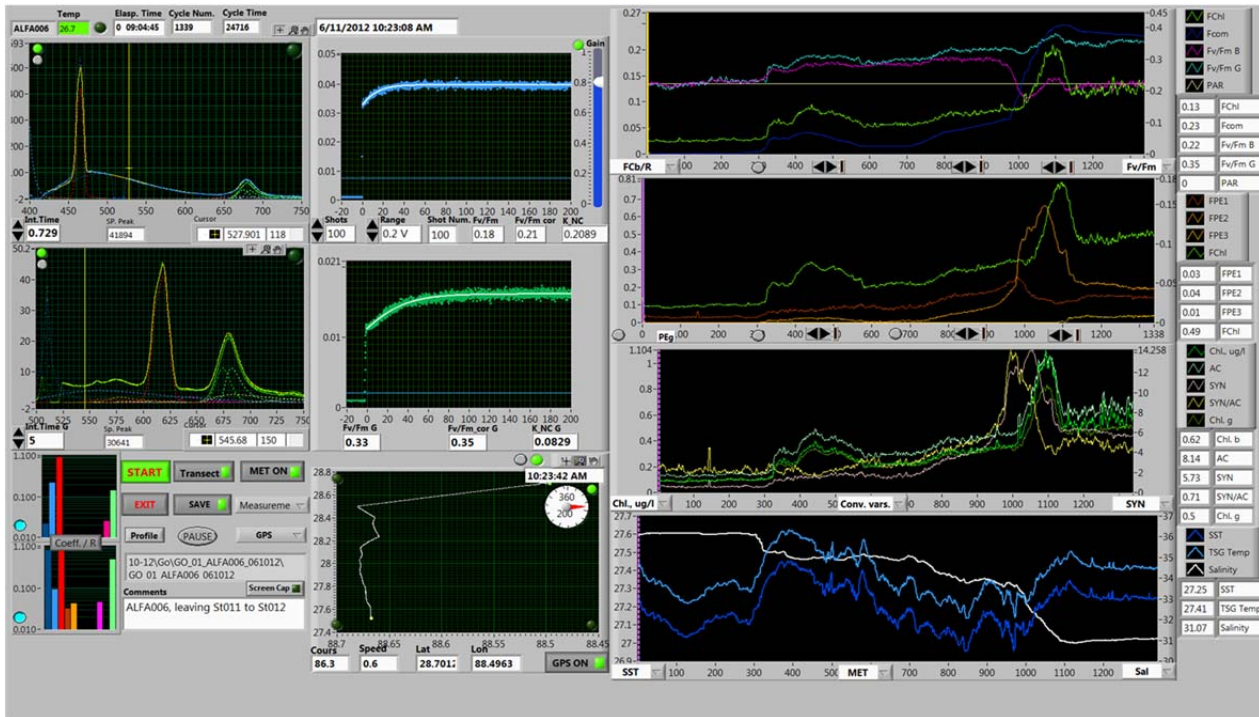
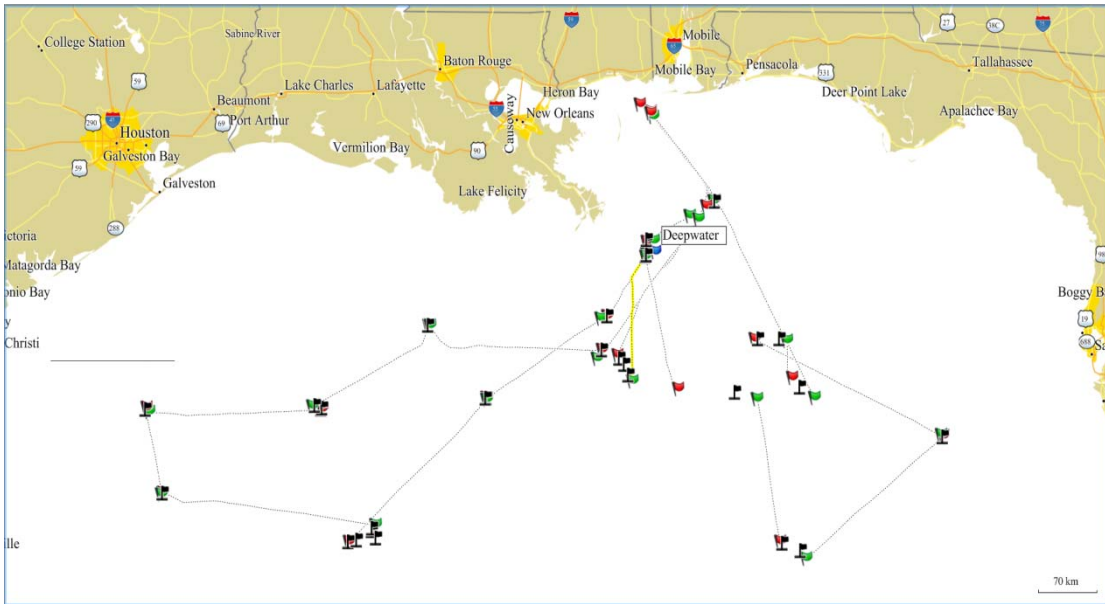
**Fig. 5. Upper: a map of transect and station ALFA measurements in the Gulf of Mexico in Apr.-May 2012. Lower: the ALFA-X-405/514 instrument upgraded with a new laser and sampling pump was tested for discrete sample analysis and underway measurements during this deployment.**



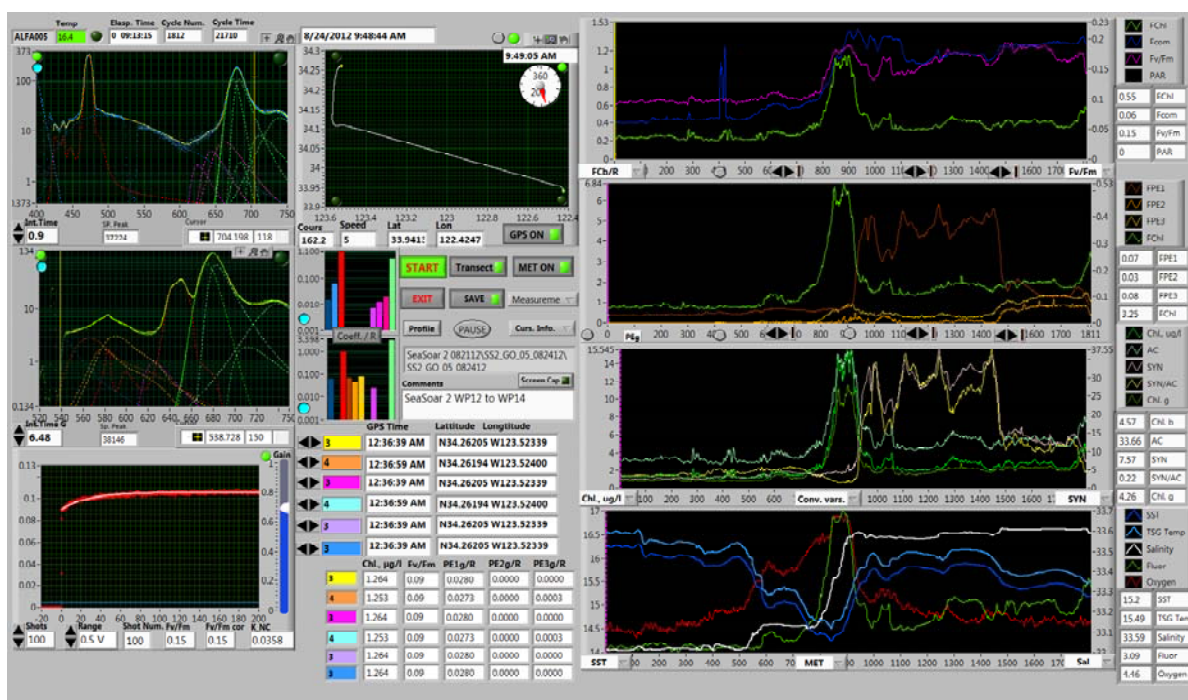
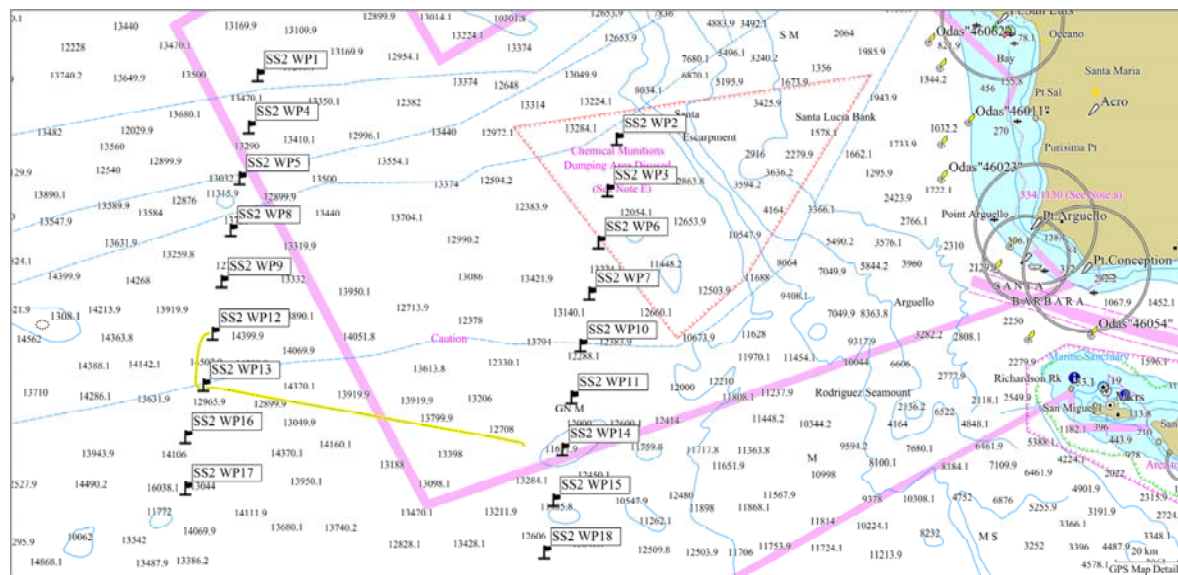
**Fig. 6. Spatial distributions of the key fluorescence characteristics measured underway in the flow-through mode by the ALFA-X-405/514 instrument. The transect track is highlighted with yellow color in the map displayed in Fig. 5. The updated ALF analytical algorithms were refined and optimized to provide accurate assessments of chlorophyll (Chl) concentration (A), CDOM (B), variable fluorescence (phytoplankton physiological characteristic, C), and basic structural characterization of phytoplankton community structure (D, E).**



**Fig. 7. (1 and 3):** Correlation between measurements of Chl concentration (Chl a,  $\text{mg m}^{-3}$ ) in discrete water samples and ALFA-X-405/514 measurements of Chl fluorescence (CF) normalized to water Raman (R) measured with 405 and 514 nm excitations (B and G, respectively). **(2 and 4):** normalizing of regular (CF/R) to variable (Fv/Fm) fluorescence provides correction of Chl fluorescence assessments for phytoplankton photo-physiological variability, including solar-induced non-photochemical quenching (Chekalyuk and Hafez, 2011) used for high-accurate underway measurements of Chl concentration (Fig. 6A). Gulf of Mexico, Apr. 2012.

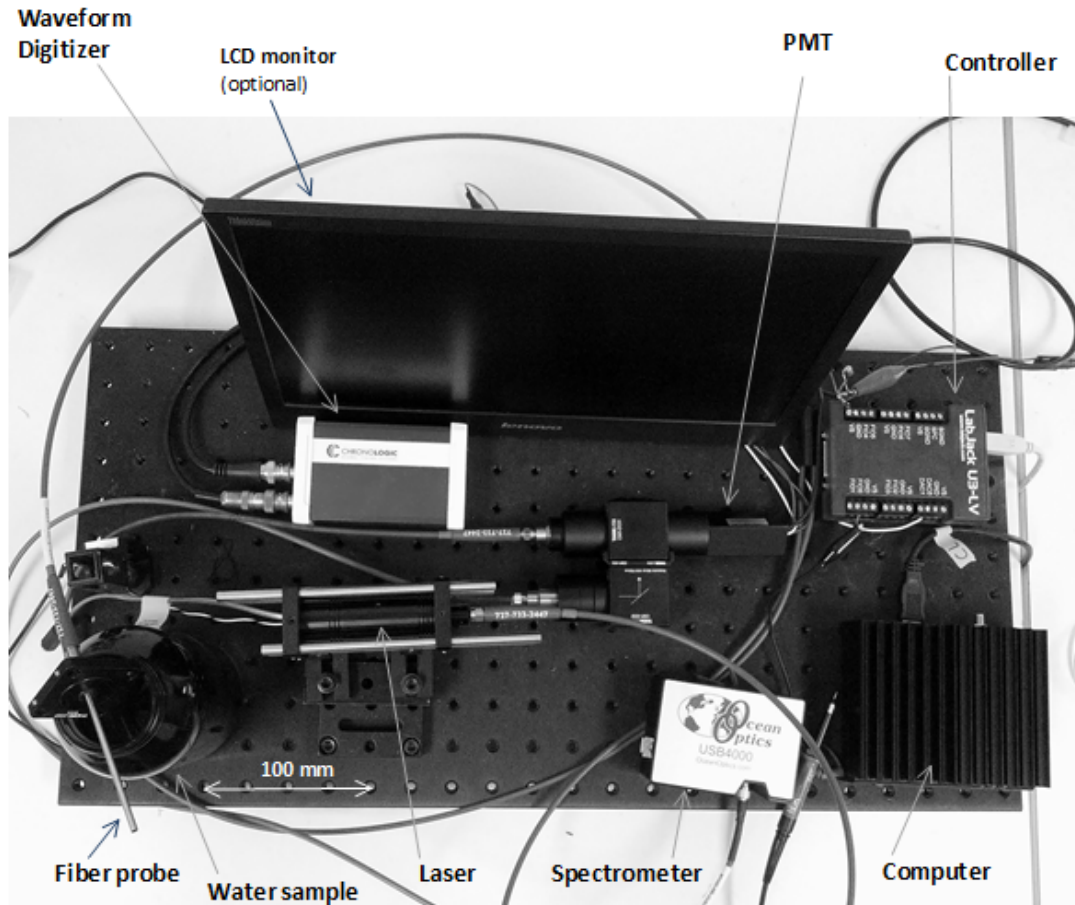


**Fig. 8.** A transect map (transect of interest highlighted in yellow) and a screenshot of the ALFA operational software taken during a cruise in the Gulf of Mexico with the ALFA instrument, May-June 2012. Right panels display spatial distributions of Chl a (green), CDOM (dark-blue, upper panel), Fv/Fm 405 nm excitation (magenta), Fv/Fm 514 nm excitation (teal), PBP pigments (yellow-orange), temperature (blue), and salinity (white).

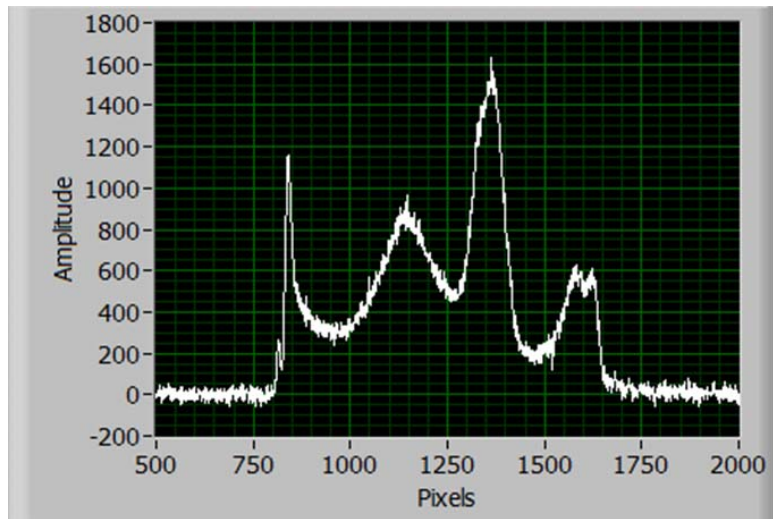
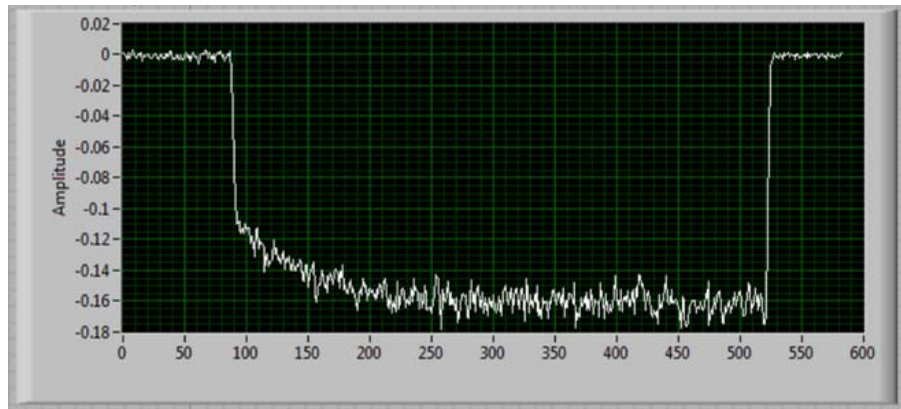


**Fig. 9.** A transect map and a screenshot of the ALFA operational software taken during the underway frontal study tow of the SeaSoar with the ALFA instrument in the California Current System, CCE LTER cruise, July-August 2012. Right panels display spatial distributions of Chl a (green), CDOM (dark-blue, upper panel), Fv/Fm (magenta), PBP pigments (yellow-orange), temperature (blue), salinity (white), and oxygen (red).

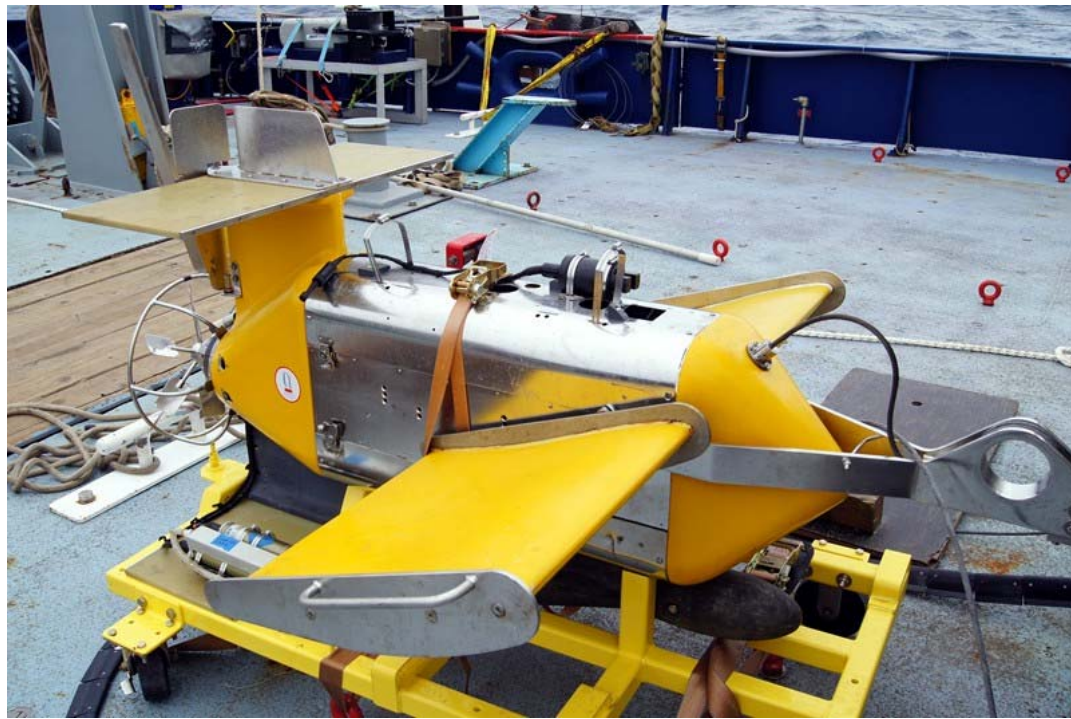




**Fig. 10. Breadboard tests of the ALF-In Situ (ALFIS) instrument prototype designed by the LDEO ALF team in June-July 2012 (provisional patent application # 2413.141PRV of July 30, 2012). The use of the new 514 nm laser, fiber-probe and dichroic beamsplitter were critical design solutions for the ALFIS prototype development. The Wet Labs and LDEO ALF project teams are currently building on this basis an operational ALFIS instrument to conduct its field tests in Spring 2013.**

**A****B**

**Fig. 11.** An example of spectrally (A) and temporally (B) resolved measurements using the ALFIS laboratory setup in Fig. 10 of the phytoplankton culture of Cryptophytes diluted with filtered seawater to natural concentration typical for estuarine waters.



*Fig. 12. The Moving Vehicle Profiler (MVP, upper photo) and the SeaSoar (lower photo) towed vehicles are considered as prospective platforms for deployments of the next generation ALF In Situ instrument.*