

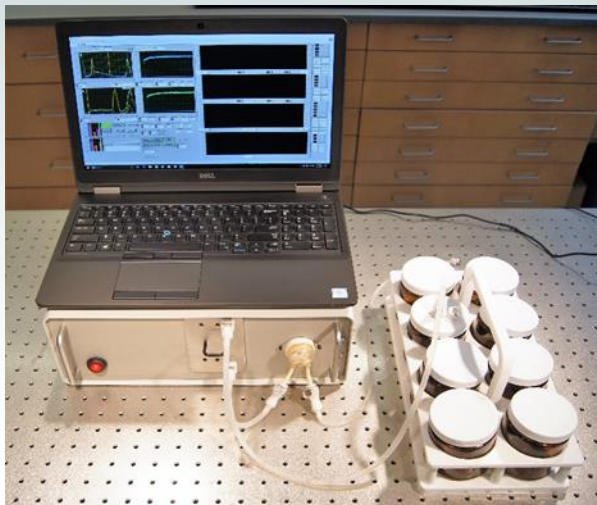
Advanced Laser Fluorometry for Characterization of Natural Aquatic Environments

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LAMONT-DOHERTY
EARTH OBSERVATORY
THE EARTH INSTITUTE AT COLUMBIA UNIVERSITY

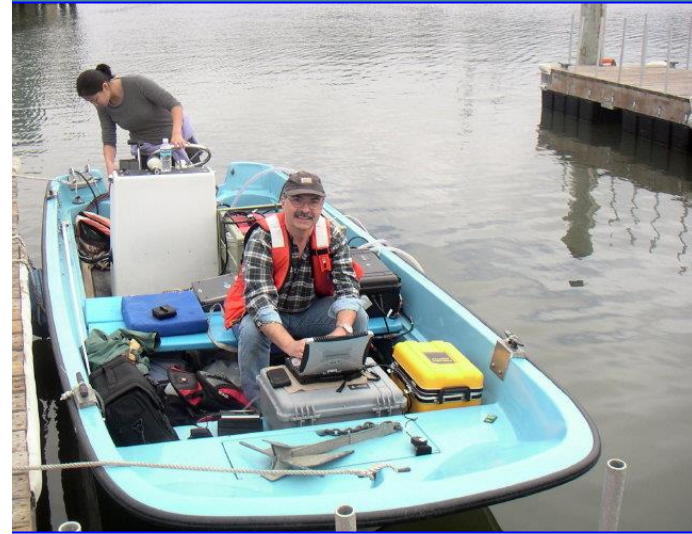
Laser Fluorescence Analysis: What We Can Do

❑ Fluorescent constituents in natural waters:

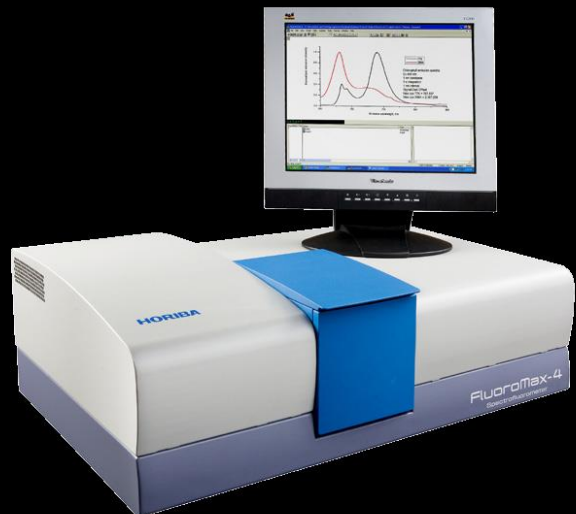
- Phytoplankton and bacteria
- Chromophoric organic matter (COM = CDOM + CPOM)
- Oil (poly-aromatic hydrocarbons (PAHs))

❑ Information that can be obtained:

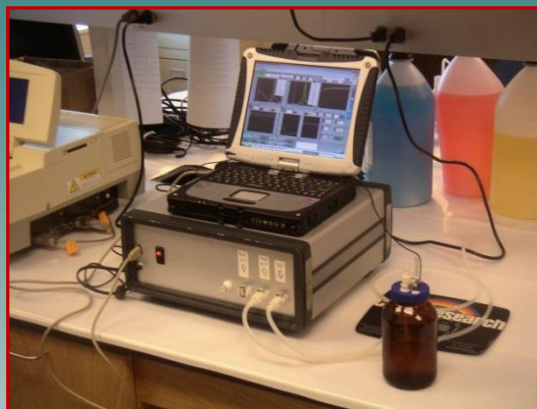
- Phytoplankton: pigments, biomass, phytoplankton composition, photo-physiology, and photosynthetic rates.
- CDOM and oil/PAHs: qualitative characterization and content assessment.



Advanced Laser Fluorometry (ALF): Filling the Gap between Laboratory and Field Instrumentation



Scanning fluorometers: provide *informative* and *data-rich* Excitation-Emission Matrix (EEM) measurements, *but* they are *bulky, slow, and not sensitive enough* for marine applications.

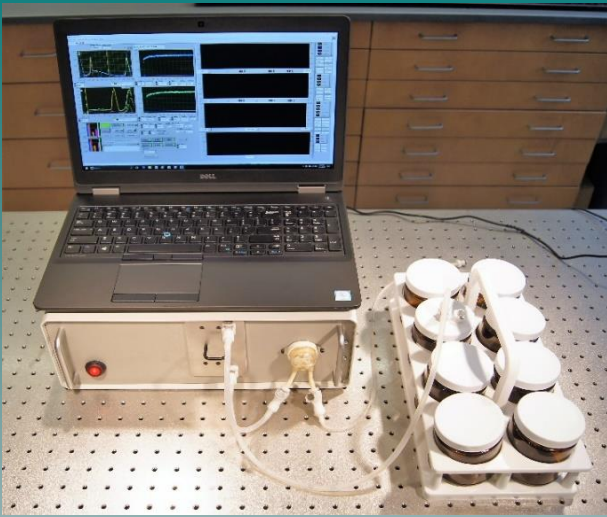


ALF – portable, field-deployable, provides *data-rich* and *accurate* characterization of natural waters.



Field fluorometers: *compact, fast, deployable,* **but** *low informative and (often) inaccurate.*

Advanced Laser Fluorometry (ALF): Motivation and Approach



Motivation:

The need for *affordable, deployable yet informative* analytical *instruments* and *methods* for characterization of natural waters.

Key solution:

Concurrent *SPECTRAL* and *TEMPORAL* measurements of *LASER*-stimulated emission.

Spectral AND Temporal Laser Fluorescence: Why?

❑ Why “laser” excitation?

- High SENSITIVITY, SELECTIVITY, and better SPECTRAL DISCRIMINATION

❑ Why “spectral” measurements? - Emission bands of aquatic fluorescence constituents are spectrally BROAD and OVERLAPPED

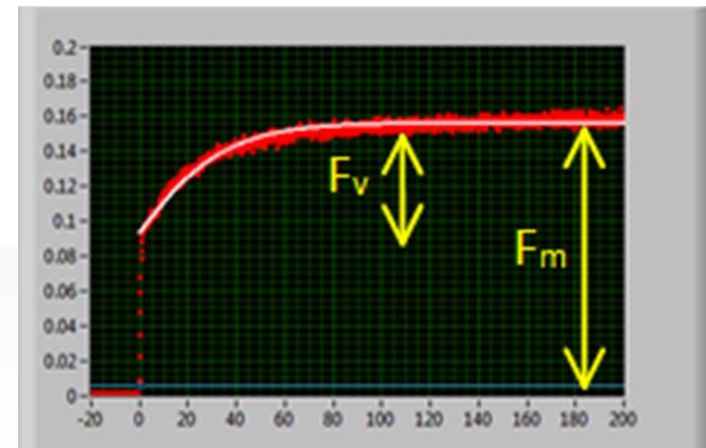
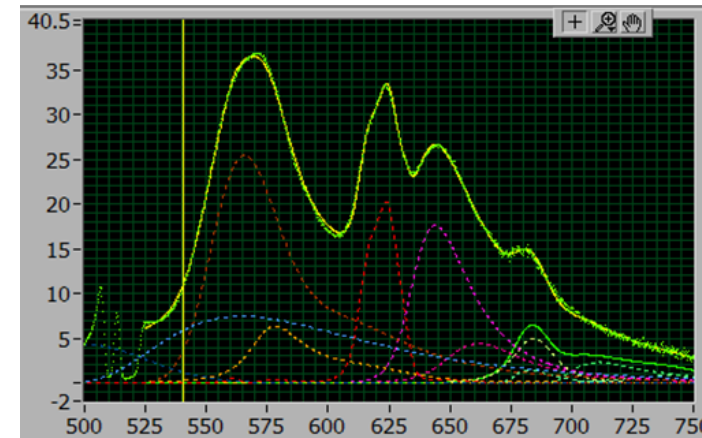
=> To address the **spectral complexity of natural waters**, we need:

- BROADBAND spectral measurements;
- Spectral DECONVOLUTION of fluorescence signatures.
- + Fluorescence normalization to water Raman also helps!

❑ Why “temporal” measurements?

- To **assess** phytoplankton PHOTO-PHYSIOLOGY (F_v/F_m) and **improve** assessments of CHLOROPHYLL

❑ Why “spectral AND temporal”? - It helps BOTH ways...



ALF: Spectral Components for SDC Analysis

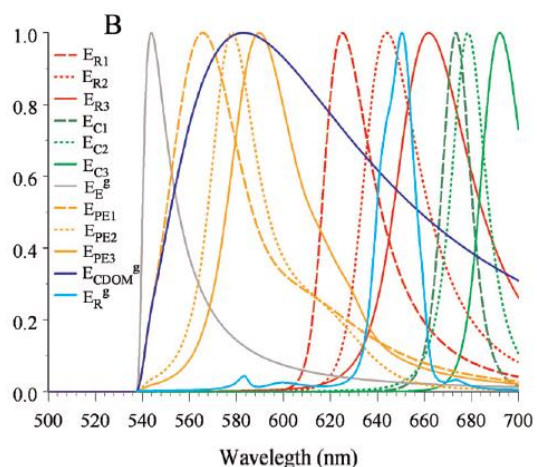
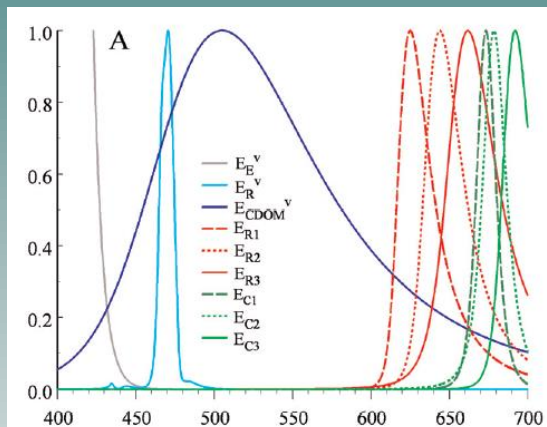
Limnol. Oceanogr.: Methods 6, 2008
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Advanced laser fluorometry of natural aquatic environments

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²EG&G Services Inc., NASA Wallops Flight Facility, Bldg. N159, Wallops Island, VA



A and B: SDC component sets for analysis of spectra measured with 405 and 532 nm excitation, respectively

$$y = \frac{a_0 \left[1 + \frac{\left(x - \frac{a_0 a_1}{2a_2} - a_1 \right)^2}{a_2^2} \right]^{-a_3} \exp \left[-a_4 \left(\tan^{-1} \left(\frac{x - \frac{a_0 a_1}{2a_2} - a_1}{a_2} \right) + \tan^{-1} \left(\frac{a_3}{2a_2} \right) \right) \right]}{\left(1 + \frac{a_4^2}{4a_2^2} \right)^{-a_3}}$$

Here, a_0 , a_1 , a_2 , a_3 , and a_4 are parameters that define the amplitude, center, width, shape₁, and shape₂, respectively, of the Pearson's IV band.

Table 1. SDC spectral components*

	Spectral component	Abbreviation	Emission peak, nm
1	Elastic scattering	E_E^v	405
2	CDOM fluorescence	E_{CDOM}^v	508
3	Water Raman scattering, 1660, 2200, and 3440 cm^{-1}	E_R^v	434, 445, 471
4	Elastic scattering	E_E^g	532
5	CDOM fluorescence	E_{CDOM}^g	587
6	Water Raman scattering, 1660, 2200, 3440 cm^{-1}	E_R^g	583, 602, 651
7	Red emission 1	E_{R1}	625
8	Red emission 2	E_{R2}	644
9	Red emission 3	E_{R3}	662
10	Chl a fluorescence	E_{C1}	673
11	Chl a fluorescence	E_{C2}	679
12	Chl a fluorescence	E_{C3}	693
13	PE fluorescence 1	E_{PE1}	565
14	PE fluorescence 2	E_{PE2}	578
15	PE fluorescence 3	E_{PE3}	589

*Three bands of the water Raman scattering with the Raman shifts $\nu_{max} = 1660, 2200, \text{ and } 3440 \text{ cm}^{-1}$, respectively, are integrated into one SDC component representing the Raman scattering in the LSE spectra. Spectral location of the individual Raman peak can be calculated as $\lambda_{max} = (\lambda_{exc}^{-1} - \nu_{max})^{-1}$; here, λ_{max} and λ_{exc} are the wavelengths of the Raman scattering peak and excitation, respectively.

ALF: SDC Analysis of Emission Spectra, 532 nm excitation

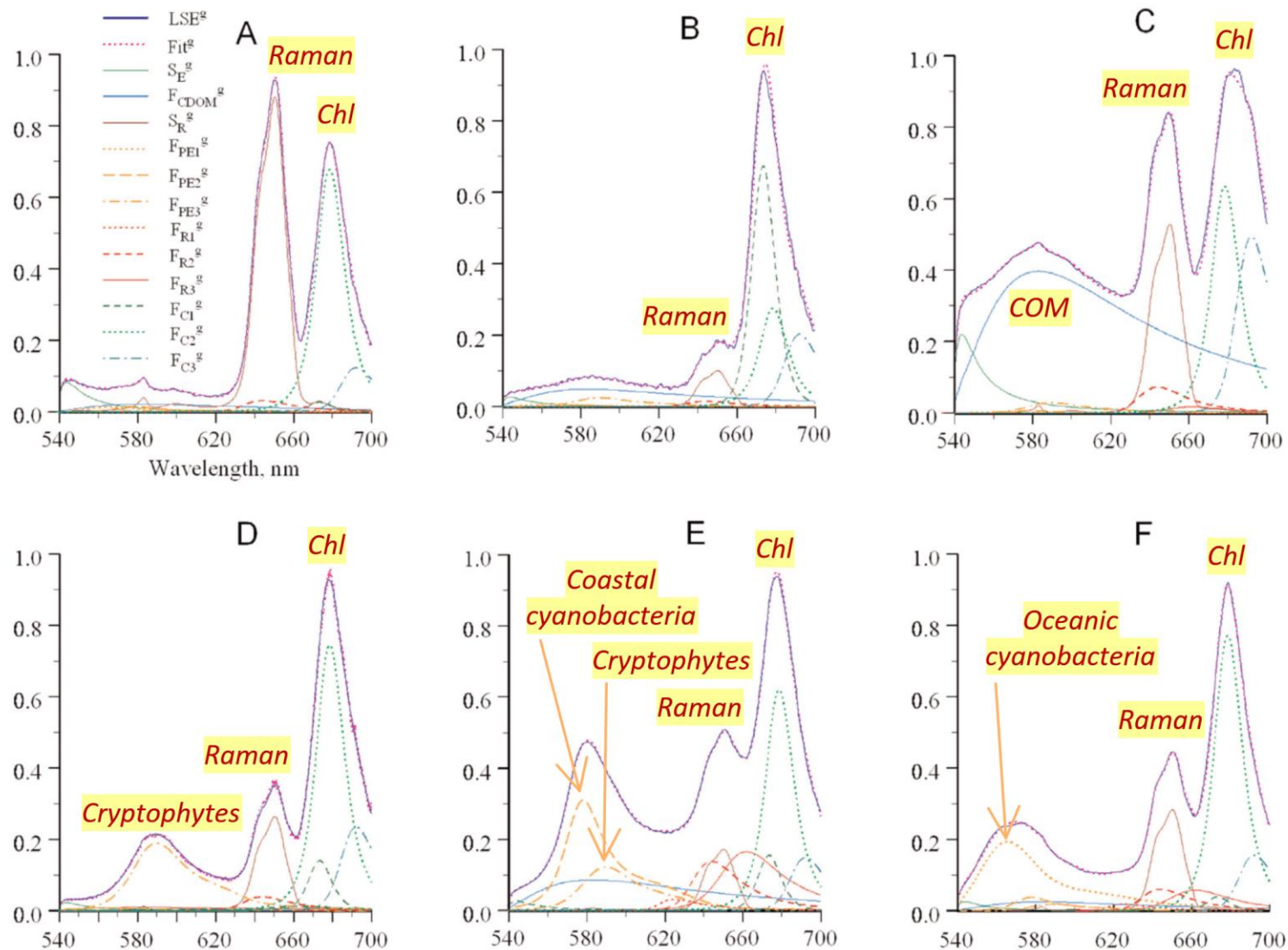
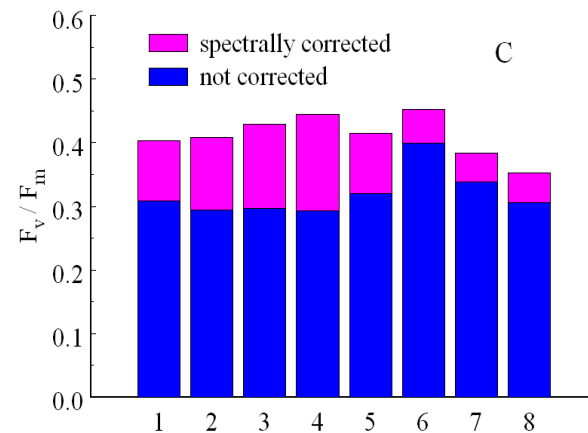
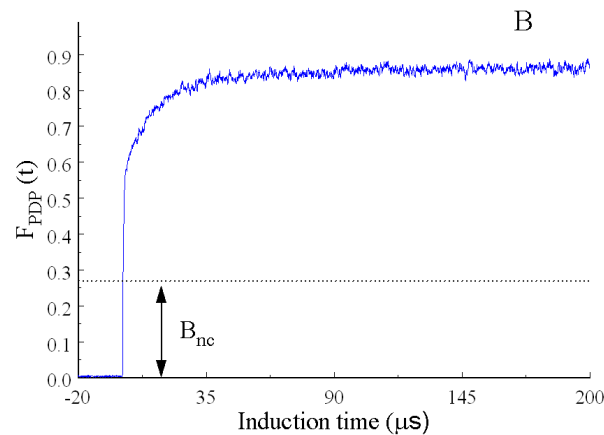
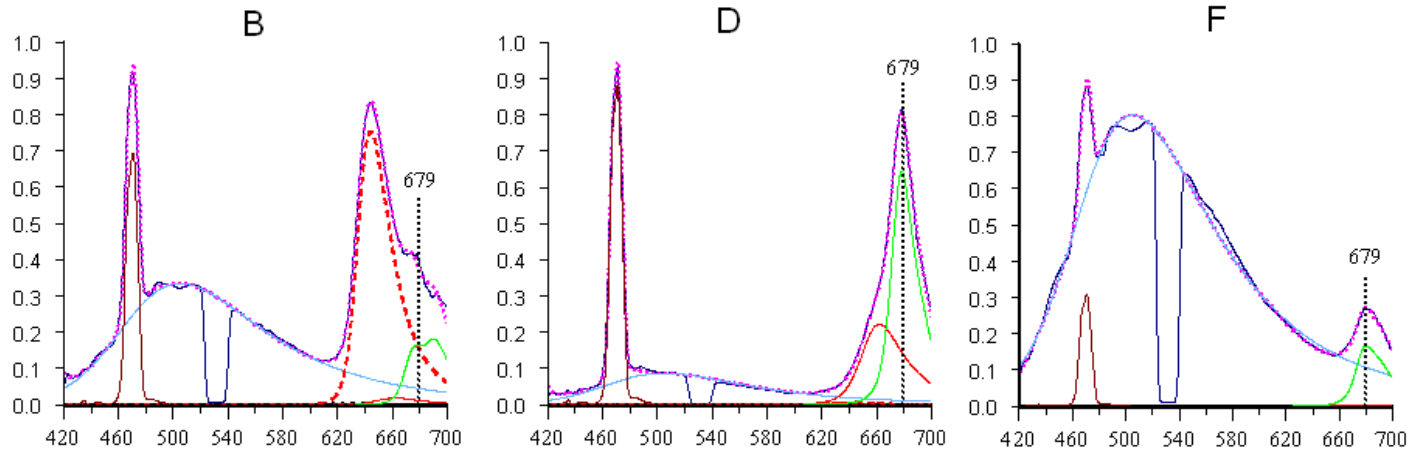


Fig. 6. Variability in the LSE⁹ spectra measured in diverse surface waters with excitation at 532 nm. (A): Southern California Bight, April 2007. (B): Moss Landing Harbor (California), September 2006. (C): Delaware River, June 2006. (D): Coastal zone of the Southern California Bight near Point Conception, April 2007. (E): Lower Chesapeake Bay, June 2005. (F): Middle Atlantic Bight, vicinity of the Delaware Bay mouth, June 2006.

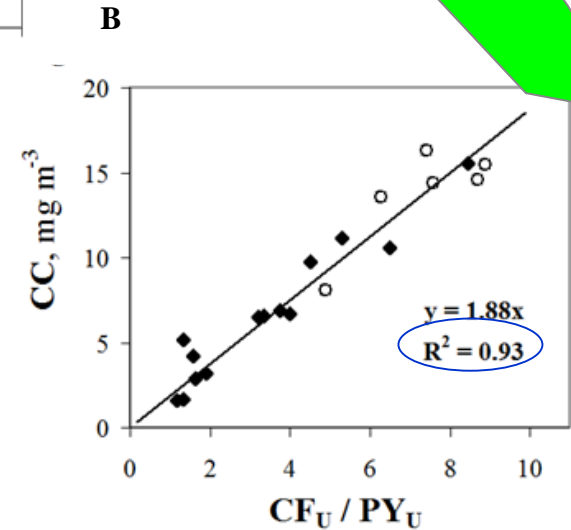
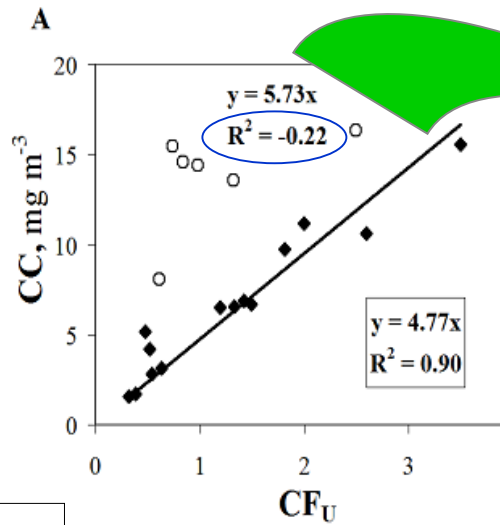
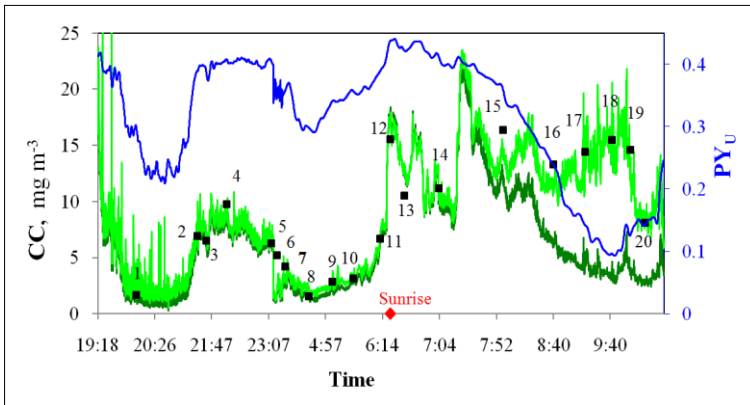
ALF: SPECTRAL measurements can help to improve TEMPORAL measurements of variable fluorescence, F_v/F_m



Upper panels: Non-Chl fluorescence in the spectral area of Chl-a fluorescence peak may be significant.

Lower panels: subtraction of none-Chl fluorescence background, B_{nc} , from fluorescence induction measurements improves the accuracy of phytoplankton photophysiological assessments, F_v/F_m .

ALF: TEMPORAL Fv/Fm measurements can help to improve SPECTRAL Chl assessments



Chekalyuk, A. and M. Hafez.
Optics Express. 19(23):
22643–22658 (2011)

(A): Correlation between the HPLC measurements of Chl concentration (CC) and the underway Chl fluorescence measurements (CF_U); the **night fluorescence measurements** (black diamonds) show **high correlation with CC** ($R^2=0.9$), while the morning, **NPQ-affected** fluorescence data (white circles) **do not correlate with CC** ($R^2= -0.22$).

(B): **normalizing CF_U to the concurrent measurements of variable fluorescence, PY_U**, can be used to eliminate the NPQ effect to **improve the accuracy of Chl fluorescence assessments**.

Initial ALF Development: NOAA/CICEET Project (2003-2006)

Advanced Laser Fluorescence (ALF) Technology for Estuarine and Coastal Environmental Biomonitoring

A Final Report Submitted to

The NOAA/UNH Cooperative Institute for Coastal and Estuarine
Environmental Technology (CICEET)

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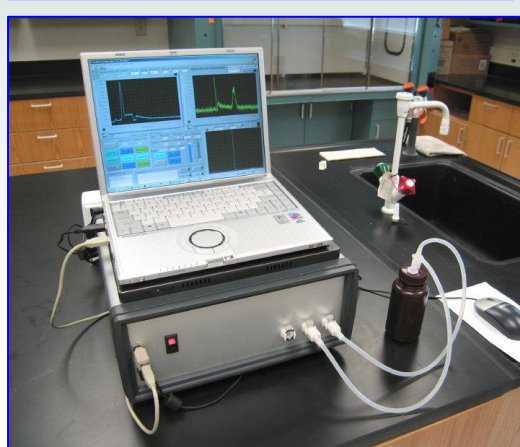
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1233 Washington Street, Columbia, SC 29201

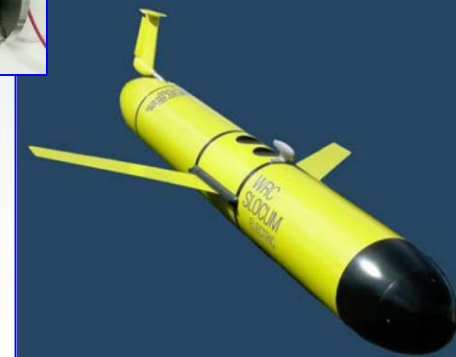
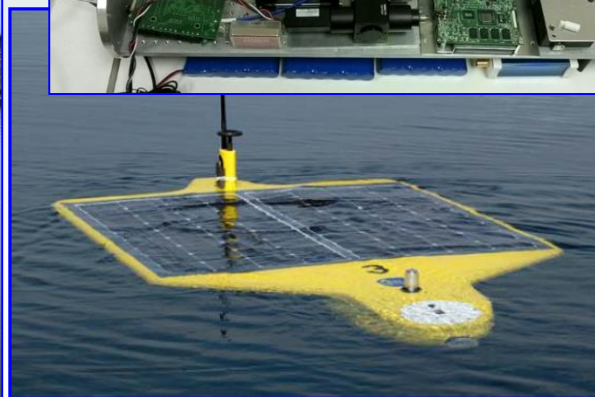
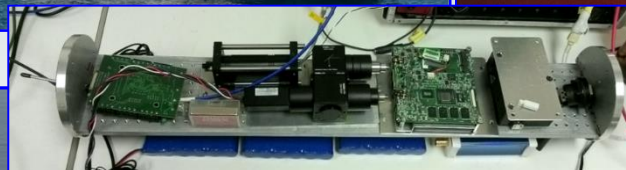
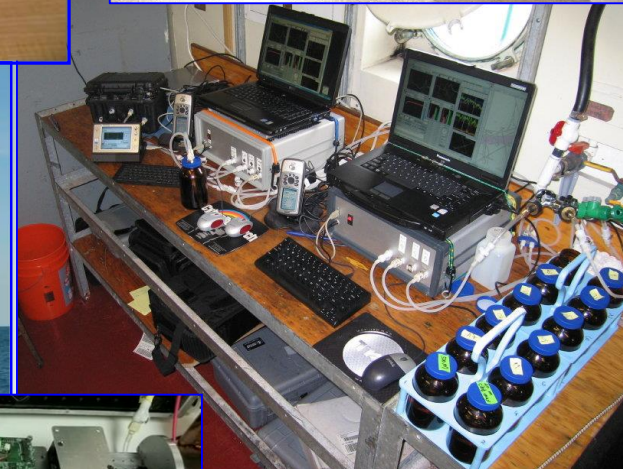
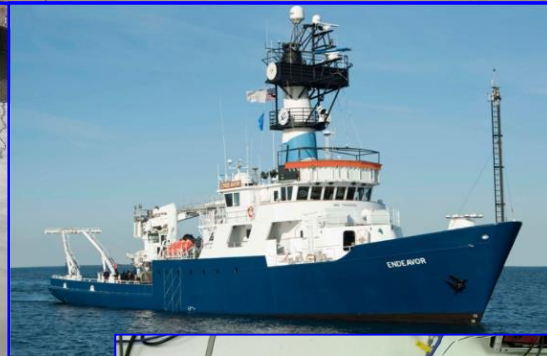
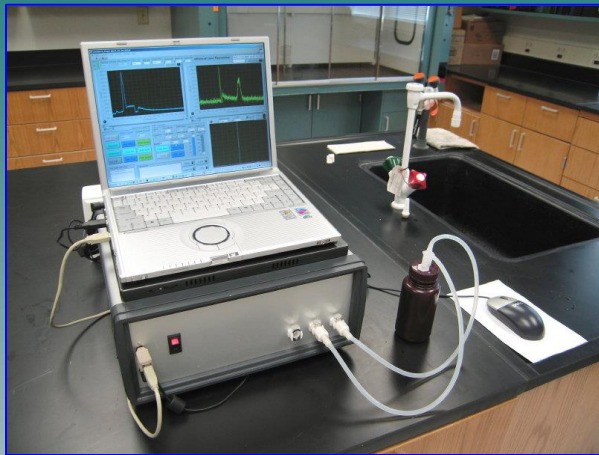
March 31, 2006



This project was funded by a grant from NOAA/UNH Cooperative Institute for Coastal and Estuarine Environmental Technology, NOAA Grant Number(s) NA03NOS4190195



ALF: Instruments and Platforms



Aquatic Laser Fluorescence Analyzer (ALFA): 1st Commercial ALF Instrument (LDEO/WET Labs)



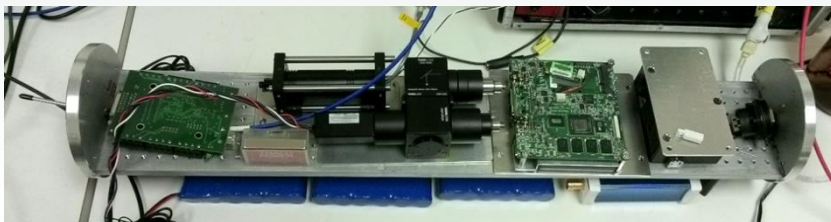
First commercial ALF instrument (ALFA) was developed by LDEO/WET Labs in 2010-2013 (sponsored by the National Oceanographic Partnership Program (NOPP))

ALFA: 405 and 514 nm laser excitation

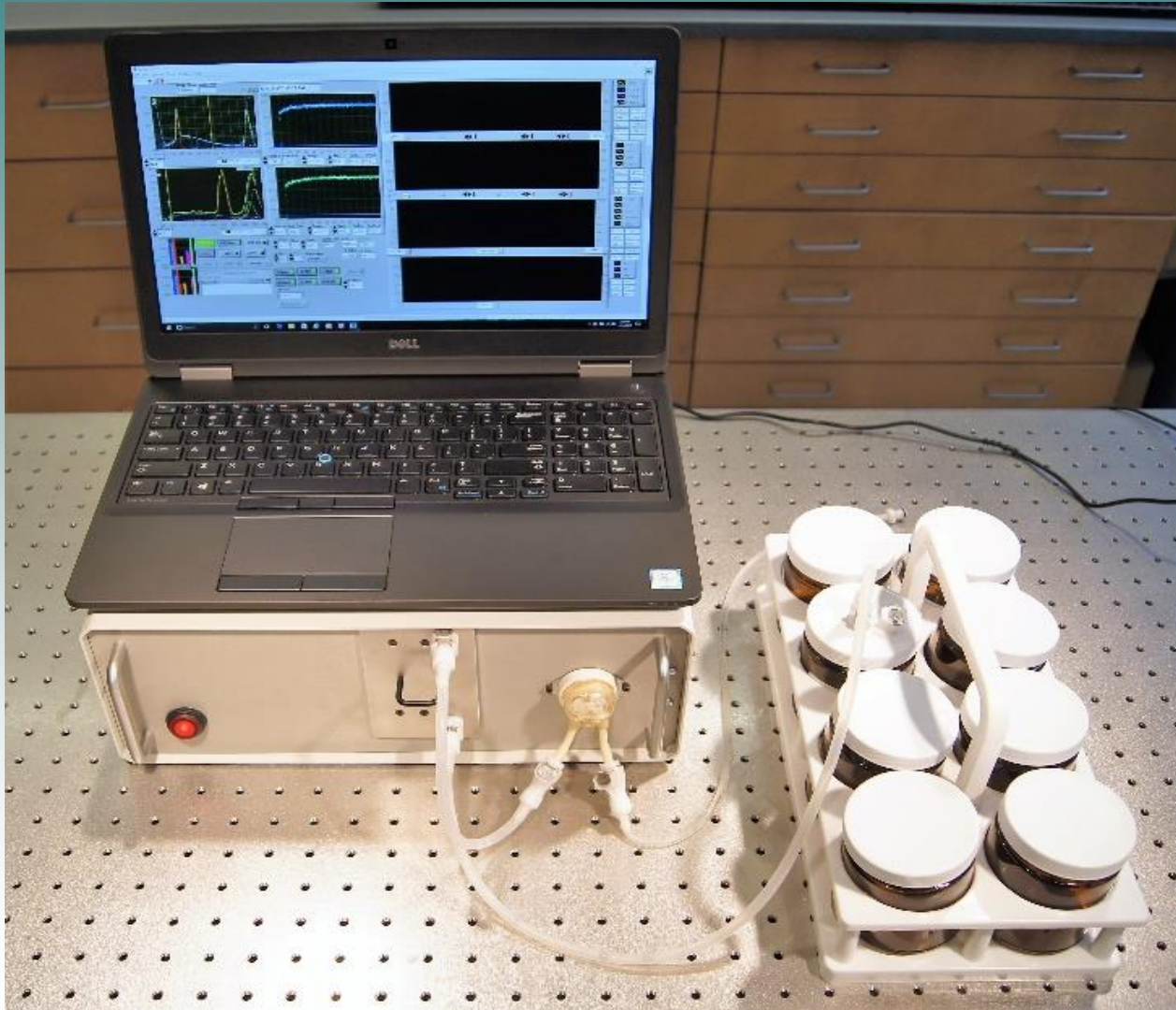
- dual laser excitation (405 and 514 nm)
- **Chlorophyll** (0.01 – 100 mg m⁻³)
- **PBP pigments** for characterization of oceanic/coastal **cyanobacteria** and eukaryotic **cryptophytes**
- **COM** (CDOM + CPOM)
- Phytoplankton photochemical efficiency, **Fv/Fm**

ALF In Situ (ALFIS) prototype: 514 nm laser excitation

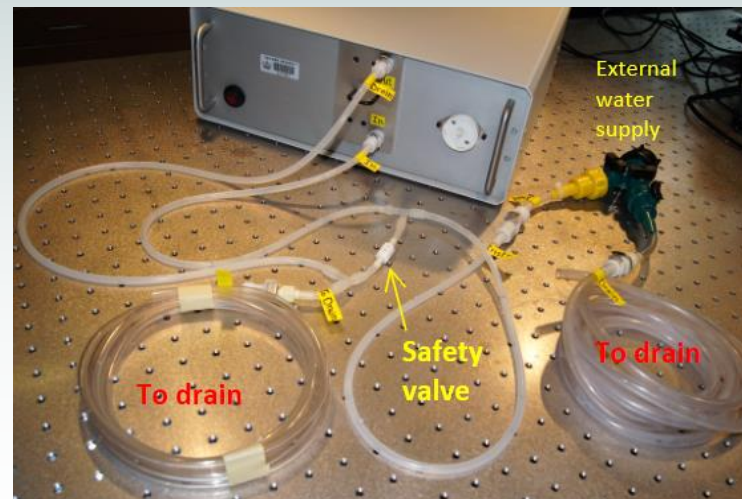
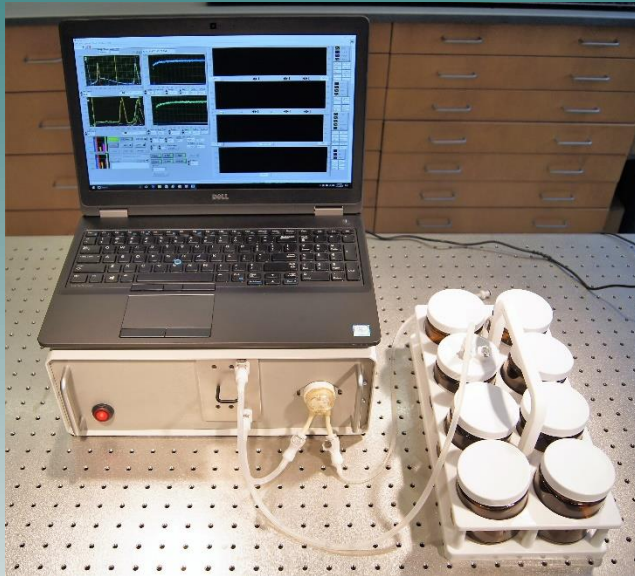
- **Chl** and **PBP pigments** for characterization of **blue/green-water cyanobacteria** and eukaryotic **cryptophytes, Fv/Fm, and CDOM** (coastal/estuarine/fresh waters)



Next Generation ALF: Custom Laser Analytical Spectroscopic System (CLASS)



Next Generation ALF: Custom Laser Analytical Spectroscopic Systems (CLASS)



ALF Methods and Instrument Designs: Two Broad US Patents (2015 & 2017)

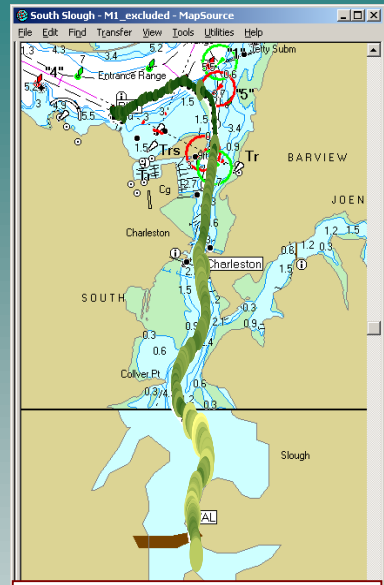
(12) United States Patent Chekalyuk		(10) Patent No.: US 8,970,841 B2
		(45) Date of Patent: Mar. 3, 2015
(54) SPECTRAL AND TEMPORAL LASER FLUORESCENCE ANALYSIS SUCH AS FOR NATURAL AQUATIC ENVIRONMENTS	(56) References Cited	
	U.S. PATENT DOCUMENTS	
(75) Inventor: Alexander Chekalyuk , Bridgewater, NJ (US)	4,084,905 A 4/1978 Schreiber et al. 4,293,225 A 10/1981 Wheaton et al.	
(73) Assignee: The Trustees of Columbia University in the City of New York , New York, NY (US)	(Continued)	
(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 141 days.	FOREIGN PATENT DOCUMENTS	
(21) Appl. No.: 13/513,786	CN 102753959 A 10/2012	
(22) PCT Filed: Dec. 3, 2010	WO 2010058891 A1 5/2010	
(86) PCT No.: PCT/US2010/058891	WO 2011069067 A1 6/2011	
§ 371 (c)(1), (2), (4) Date: Sep. 11, 2012	OTHER PUBLICATIONS	
(87) PCT Pub. No.: WO2011/069067	"CICEET Progress Report for the period Sep. 15, 2004 Through Mar. 15, 2005; dvanced Laser Fluorescence (ALF) Technology for Estuarine and Coastal Environmental Biomonitoring", http://ciceet.unh.edu/progressreports/2005/3_2005/chekalyuk2003/ , 6 pgs.	
PCT Pub. Date: Jun. 9, 2011	(Continued)	
(65) Prior Publication Data	<i>Primary Examiner</i> — Kara E Geisel	
US 2012/0324986 A1 Dec. 27, 2012	<i>Assistant Examiner</i> — Hina F Ayub	
Related U.S. Application Data	(74) <i>Attorney, Agent, or Firm</i> — Schwegman Lundberg & Woessner, P.A.	
(60) Provisional application No. 61/266,756, filed on Dec. 4, 2009.	(57) ABSTRACT	
(51) Int. Cl. G01J 3/30 (2006.01) G01J 3/28 (2006.01) (Continued)	An Advanced Laser Fluorometer (ALF) can combine spectrally and temporally resolved measurements of laser-stimulated emission (LSE) for characterization of dissolved and particulate matter, including fluorescence constituents, in liquids. Spectral deconvolution (SDC) analysis of LSE spectral measurements can accurately retrieve information about individual fluorescent bands, such as can be attributed to chlorophyll-a (Chl-a), phycobiliprotein (PBP) pigments, or chromophoric dissolved organic matter (CDOM), among others. Improved physiological assessments of photosynthesizing organisms can use SDC analysis and temporal LSE measurements to assess variable fluorescence corrected for SDC-retrieved background fluorescence. Fluorescence assessments of Chl-a concentration based on LSE spectral measurements can be improved using photo-physiological information from temporal measurements. Quantitative assessments of PBP pigments, CDOM, and other fluorescent constituents, as well as basic structural characterizations of photosynthesizing populations, can be performed using SDC analysis of LSE spectral measurements.	
(52) U.S. Cl. CPC G01N 21/6408 (2013.01); G01J 3/021 (2013.01); G01J 3/0264 (2013.01); G01J 3/4406 (2013.01); (Continued)	25 Claims, 23 Drawing Sheets	
(58) Field of Classification Search None See application file for complete search history.		

(12) United States Patent Chekalyuk		(10) Patent No.: US 9,618,449 B2
		(45) Date of Patent: Apr. 11, 2017
(54) OPTICAL ANALYSIS OF EMISSIONS FROM STIMULATED LIQUIDS	(52) U.S. Cl. CPC G01N 21/6402 (2013.01); G01J 3/021 (2013.01); G01J 3/0208 (2013.01); (Continued)	
(71) Applicant: The Trustees of Columbia University in the City of New York , New York, NY (US)	(58) Field of Classification Search CPC .. G02B 26/0833 ; G02B 26/08 ; G02B 26/085 ; G02B 26/0875 ; G02B 26/10 ; (Continued)	
(72) Inventor: Alexander Chekalyuk , Bridgewater, NJ (US)	(56) References Cited	
(73) Assignee: The Trustees of Columbia University in the City of New York , New York, NY (US)	U.S. PATENT DOCUMENTS	
(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 120 days.	4,084,905 A 4/1978 Schreiber et al. 4,144,452 A 3/1979 Harie (Continued)	
(21) Appl. No.: 14/376,297	FOREIGN PATENT DOCUMENTS	
(22) PCT Filed: Feb. 1, 2013	WO WO-2011069067 A1 6/2011 WO WO-2013116760 A1 8/2013	
(86) PCT No.: PCT/US2013/024484	OTHER PUBLICATIONS	
§ 371 (c)(1), (2) Date: Aug. 1, 2014	"International Application Serial No. PCT/US2013/024484, International Search Report mailed Apr. 22, 2013", 3 pgs. (Continued)	
(87) PCT Pub. No.: WO2013/116760	<i>Primary Examiner</i> — Michael P Stafira	
PCT Pub. Date: Aug. 8, 2013	(74) <i>Attorney, Agent, or Firm</i> — Schwegman, Lundberg & Woessner, P.A.	
(65) Prior Publication Data	(57) ABSTRACT	
US 2015/0000384 A1 Jan. 1, 2015	Modular systems can be used for optical analysis, including in-situ analysis, of stimulated liquids. An excitation module can include a radiation sources, e.g., a laser, LED, lamp, etc. A detection module can include one or more detectors configured to receive spectral and/or temporal information from a stimulated liquid. Such systems can be used to identify or measure optical emissions including fluorescence or scattering. The efficient excitation of liquid samples and collection of emissions from the samples provides substantial, up to four-fold increase in the emission signal over prior systems. In an example, emission measurements can be	
Related U.S. Application Data	(Continued)	
(60) Provisional application No. 61/594,778, filed on Feb. 3, 2012, provisional application No. 61/677,318, filed on Jul. 30, 2012.		
(51) Int. Cl. G01N 21/00 (2006.01) G01N 21/64 (2006.01) (Continued)		

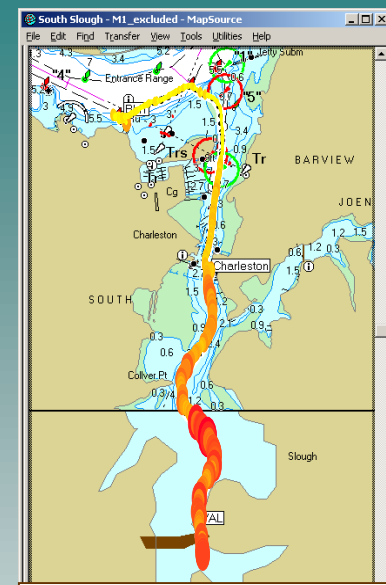
Chekalyuk, A.M. "Spectral and Temporal Laser Fluorescence Analysis such as for Natural Aquatic Environments", **US Patent 8970841**.

Chekalyuk, A.M. "Optical Analysis of Emissions from Stimulated Liquids", **US Patent 9618449**.

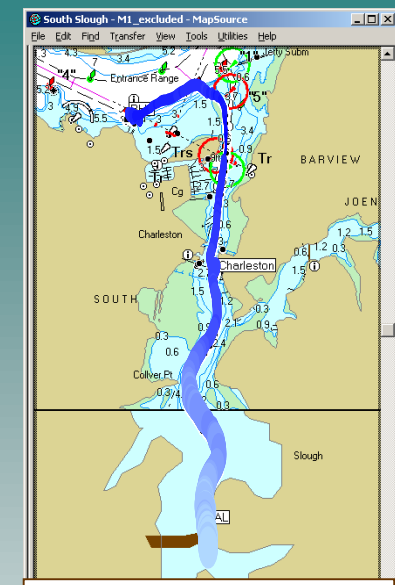
ALF: Motorboat underway survey at Sough Slow NERR (OR, 2005)



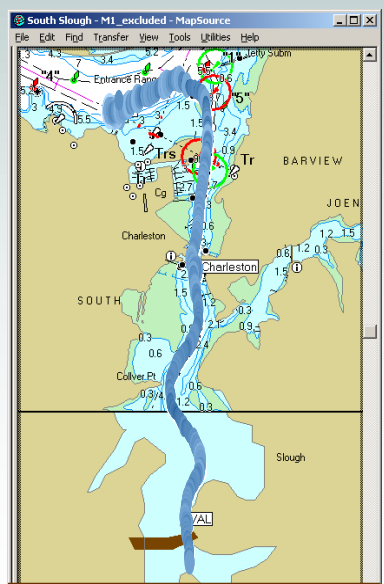
Chlorophyll



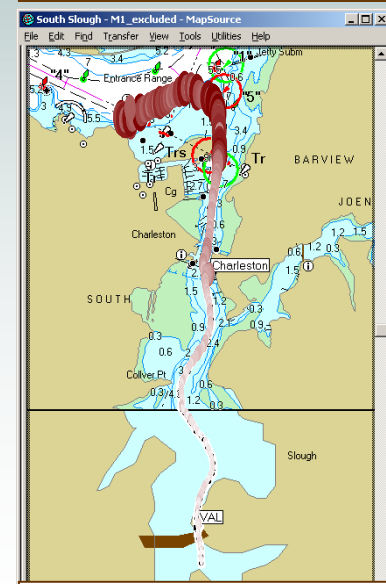
Phycoerythrin



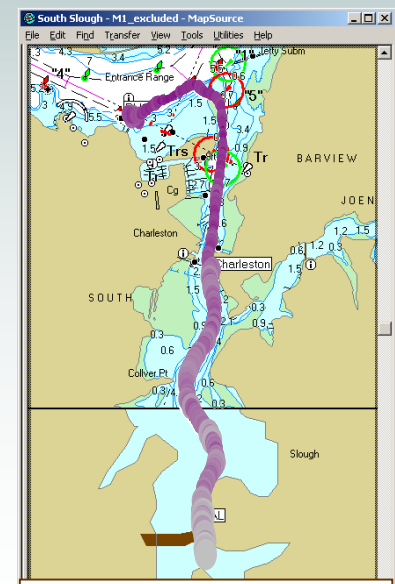
CDOM concentration



Variable fluor., Fv/Fm

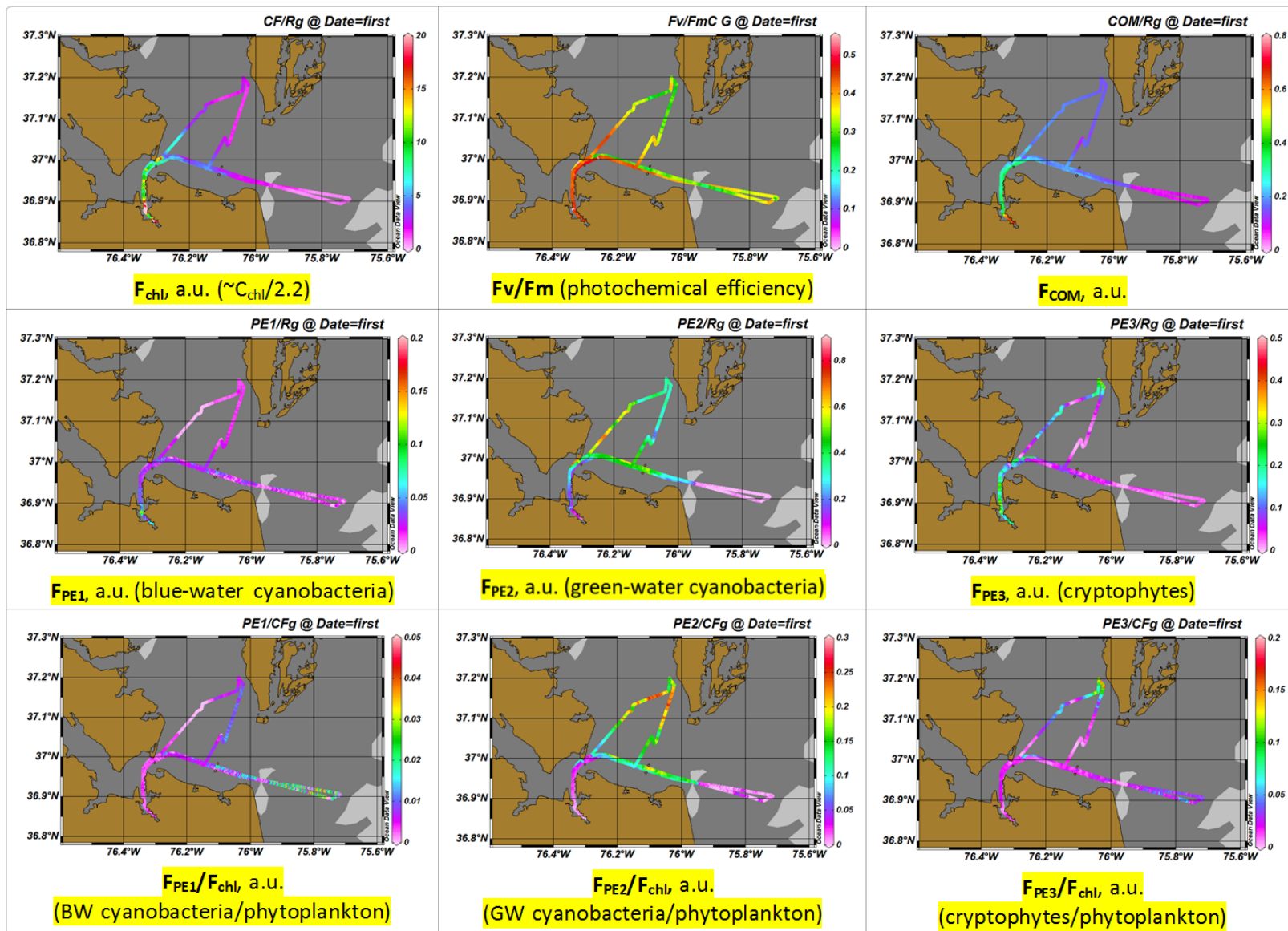


Absorption cross-section



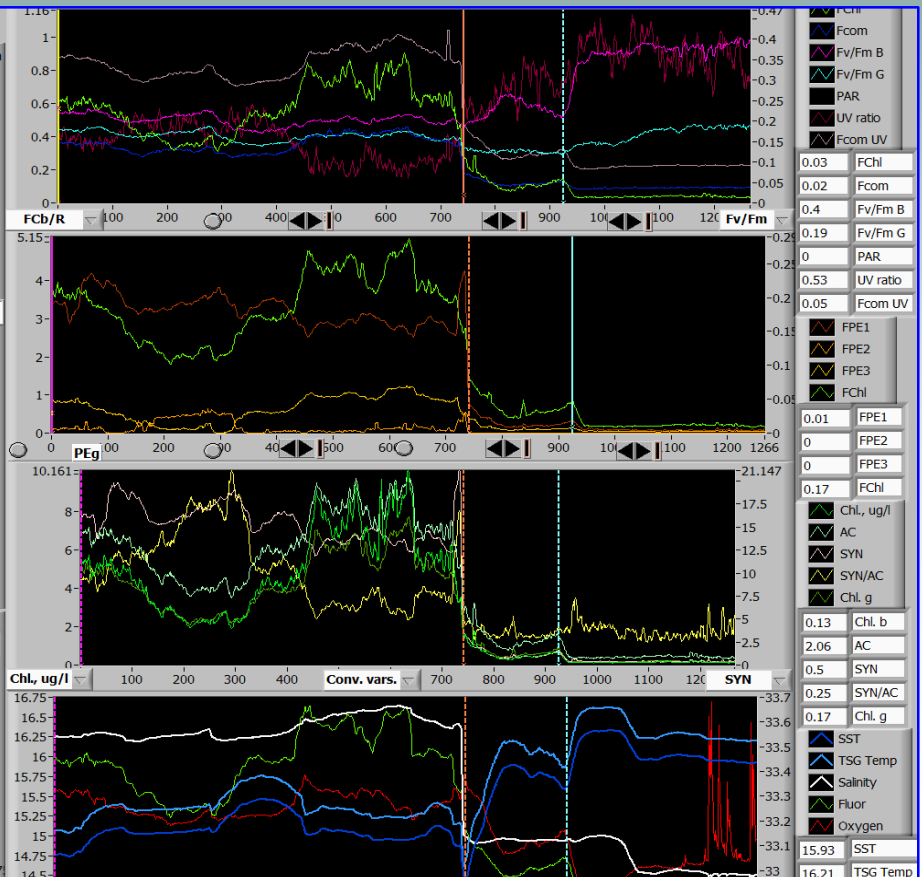
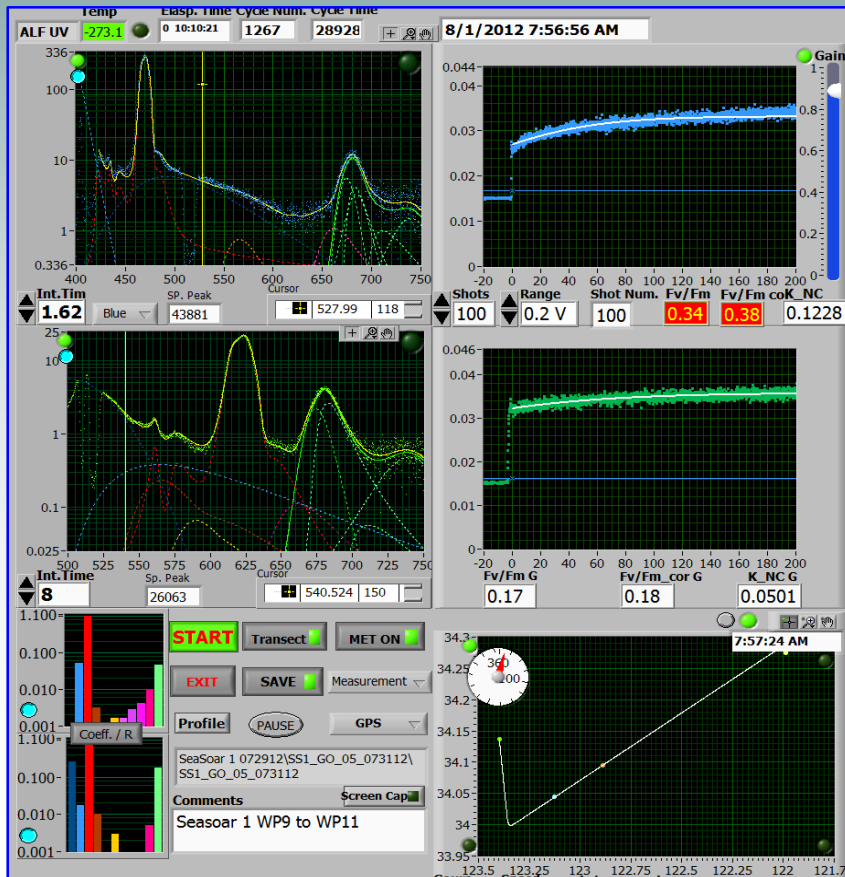
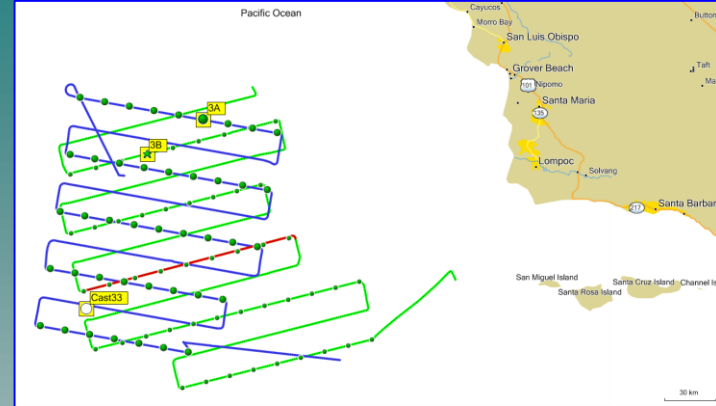
Laser elastic scattering

ALFA: Underway measurements in Chesapeake Bay

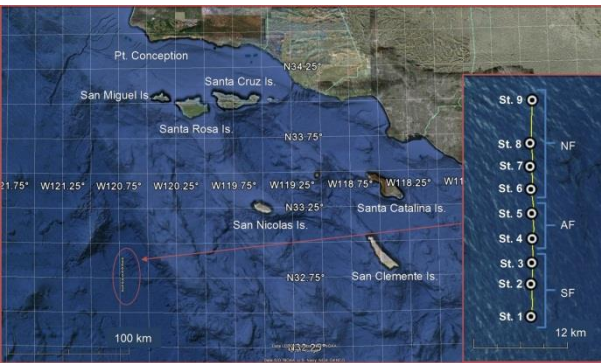


(Aug. 2013; measured by Gilerson et al.)

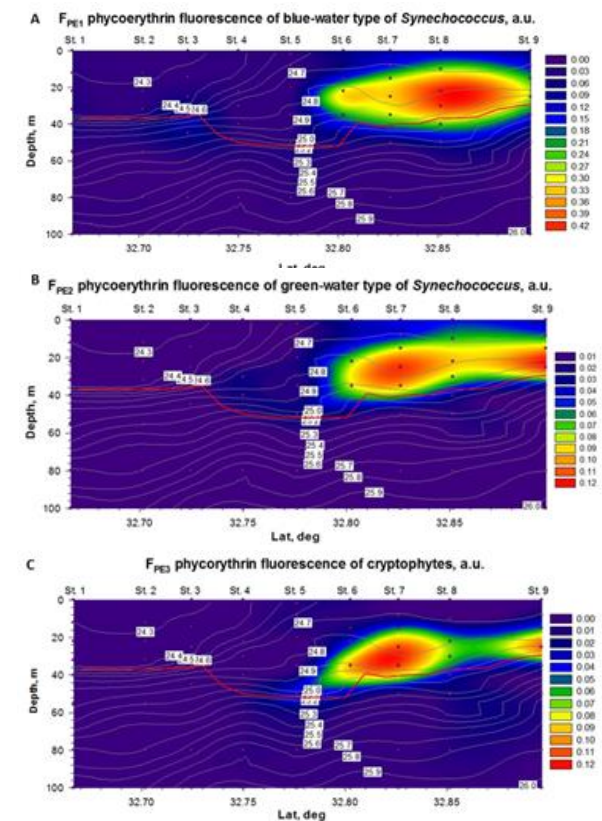
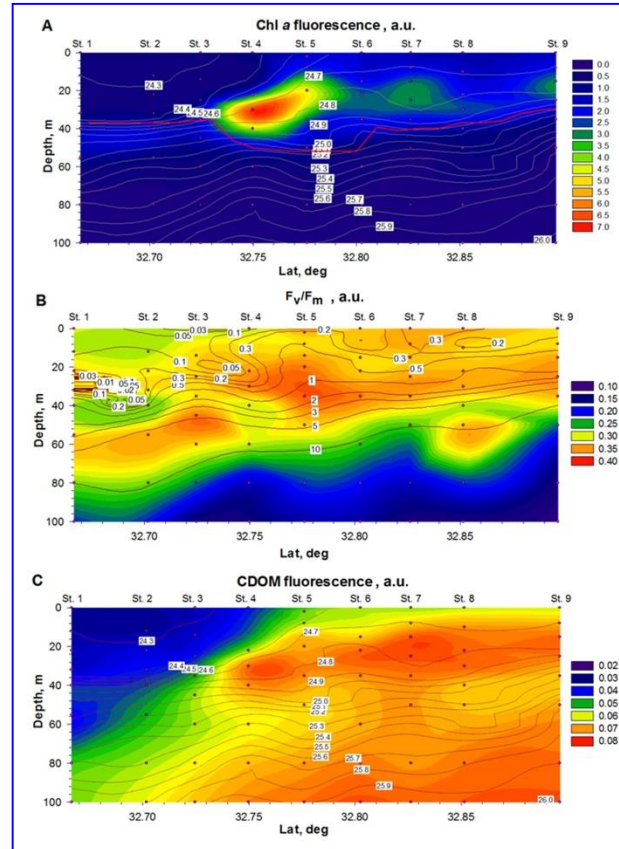
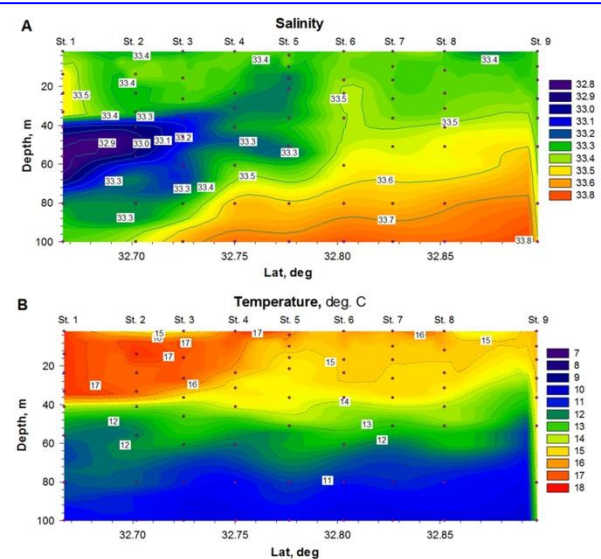
ALF: Mapping biological responses across oceanic fronts



ALF: Variability in phytoplankton biomass, physiology, structure and COM across the oceanic fronts



Chekalyuk et al., Laser fluorescence analysis of phytoplankton across a frontal zone in the California Current ecosystem, *J. Plankton Res.*, **34**(9), 761-777 (2012)

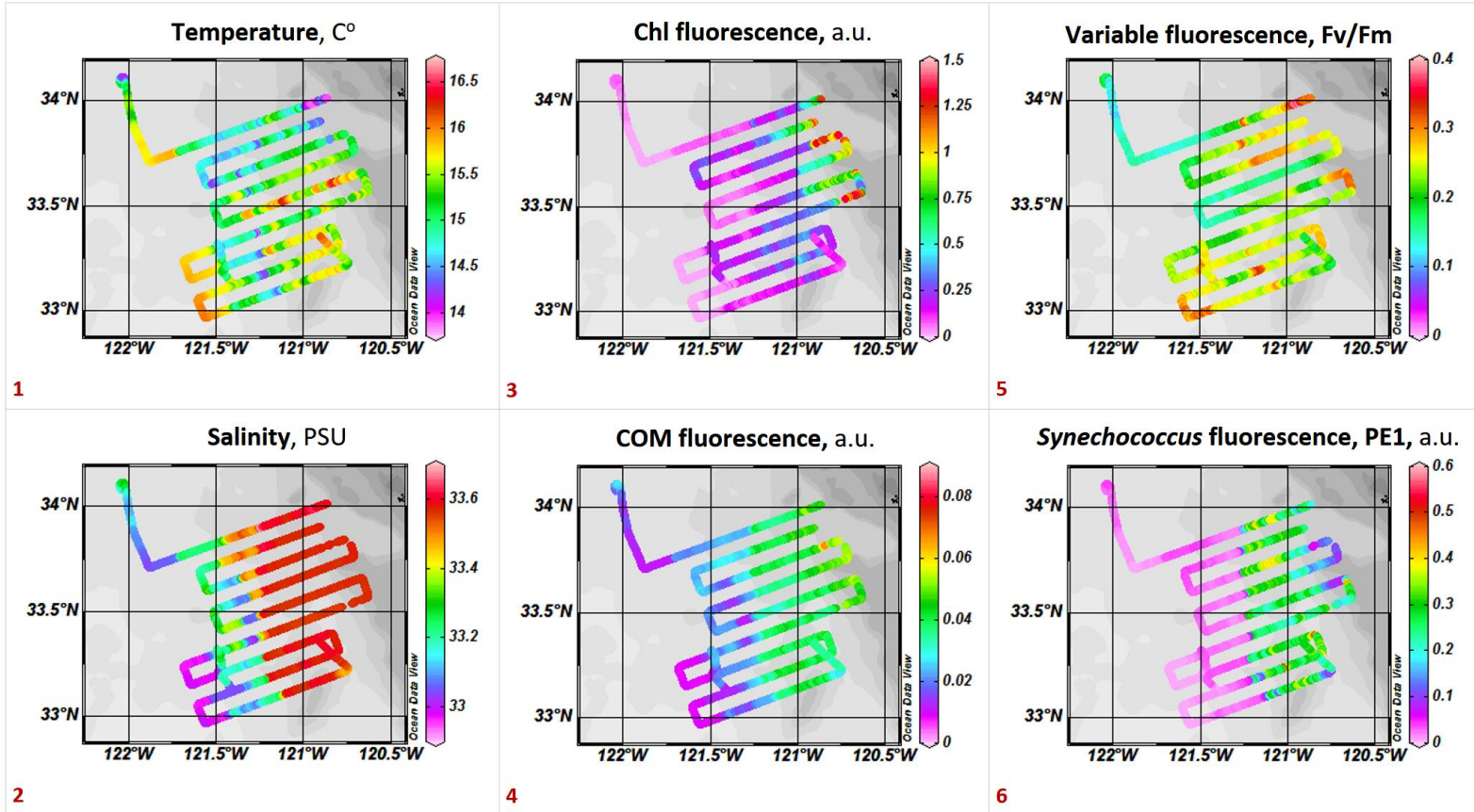


Distributions of seawater salinity (A) and temperature (B) in the euphotic layer across the A-front.

Spatial distributions of Chl *a* (A), phytoplankton photochemical efficiency F_v/F_m (B), and CDOM (C) across the frontal zone.

Spatial distributions of blue-water (A) and green-water (B) types of cyanobacteria and cryptophytes (C) across the frontal zone.

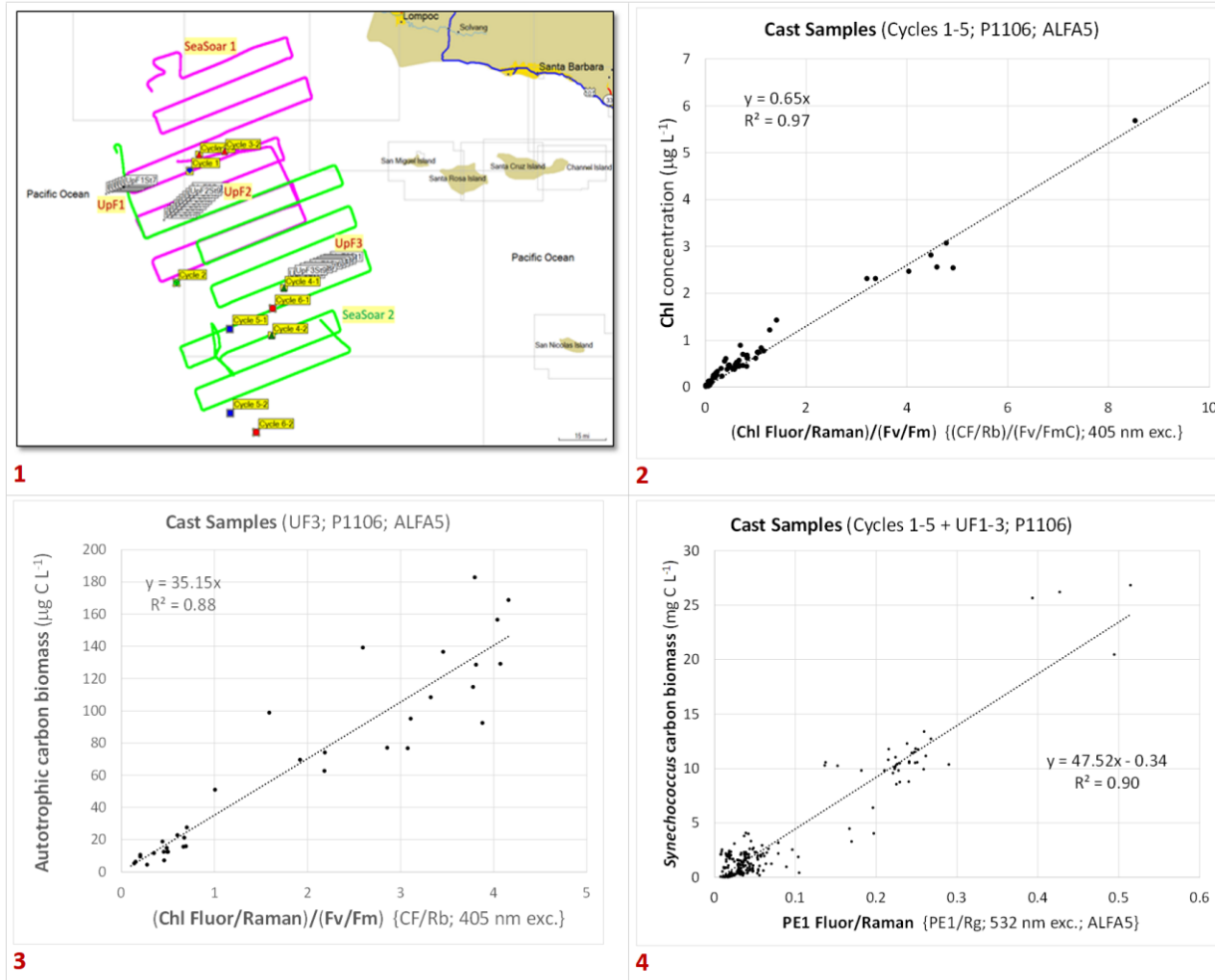
ALF: California Current Synoptic Survey (SIO, NSF), CCE LTER, 2011



Underway measurements of synoptic-scale surface distributions of sea-surface **temperature (1)**, **salinity (2)**, **Chl fluorescence (3; ALF)**, colored organic matter (**COM**) fluorescence (**4; ALF**), variable fluorescence (= phytoplankton photochemical efficiency), **Fv/Fm (5; ALF)**, and phycoerythrin fluorescence of oceanic cyanobacteria ***Synechococcus*, PE1 (6; ALF)**. California Current Ecosystem (CCE, SIO) Long-Term Ecological Research (LTER, NSF) Process cruise P1106; 3-6 July 2011.

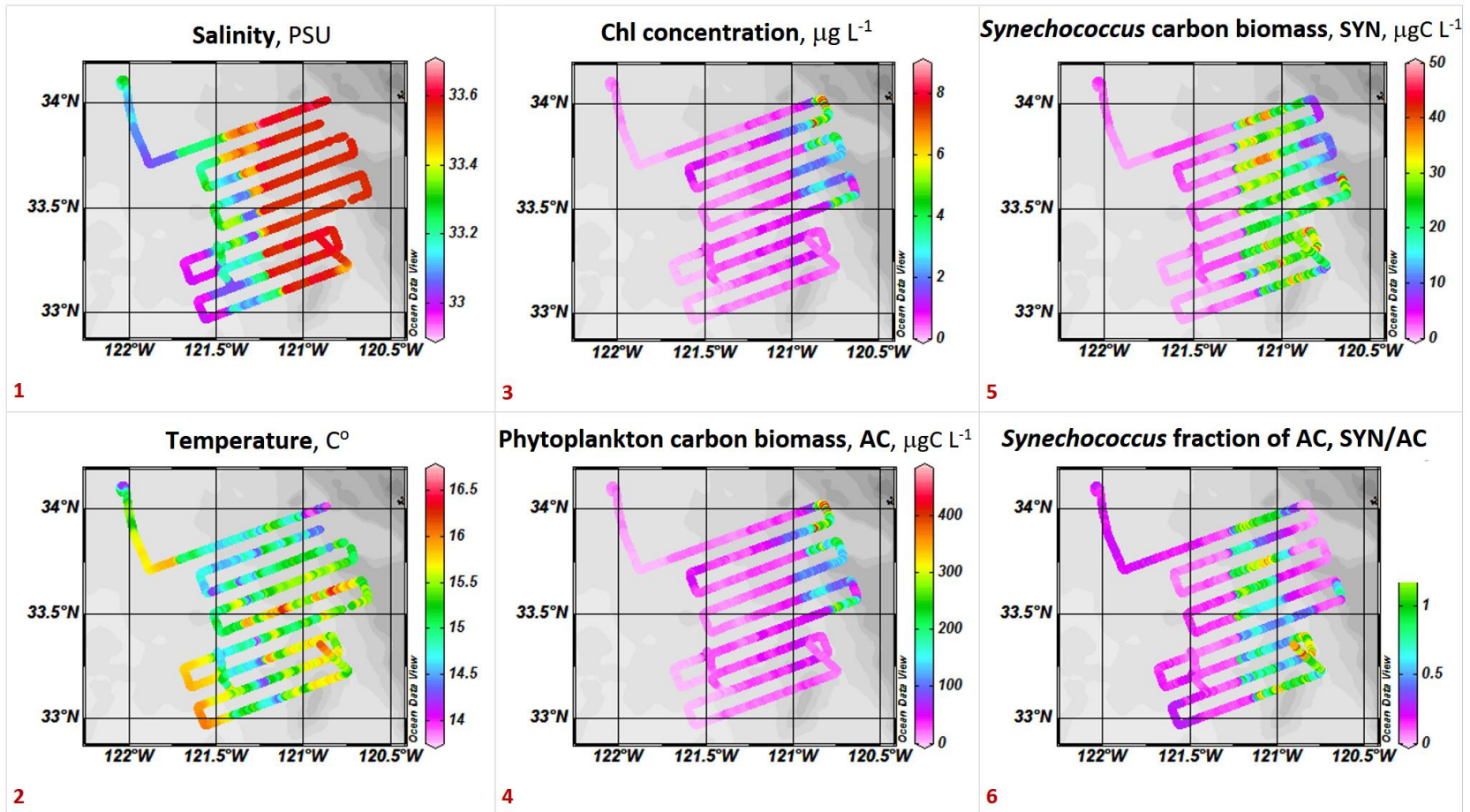
*Note: Both **Chl fluorescence** and **variable fluorescence, Fv/Fm**, were affected by solar-induced **non-photochemical quenching (NPQ)**.*

ALF: California Current Synoptic Survey (SIO, NSF), CCE LTER, 2011



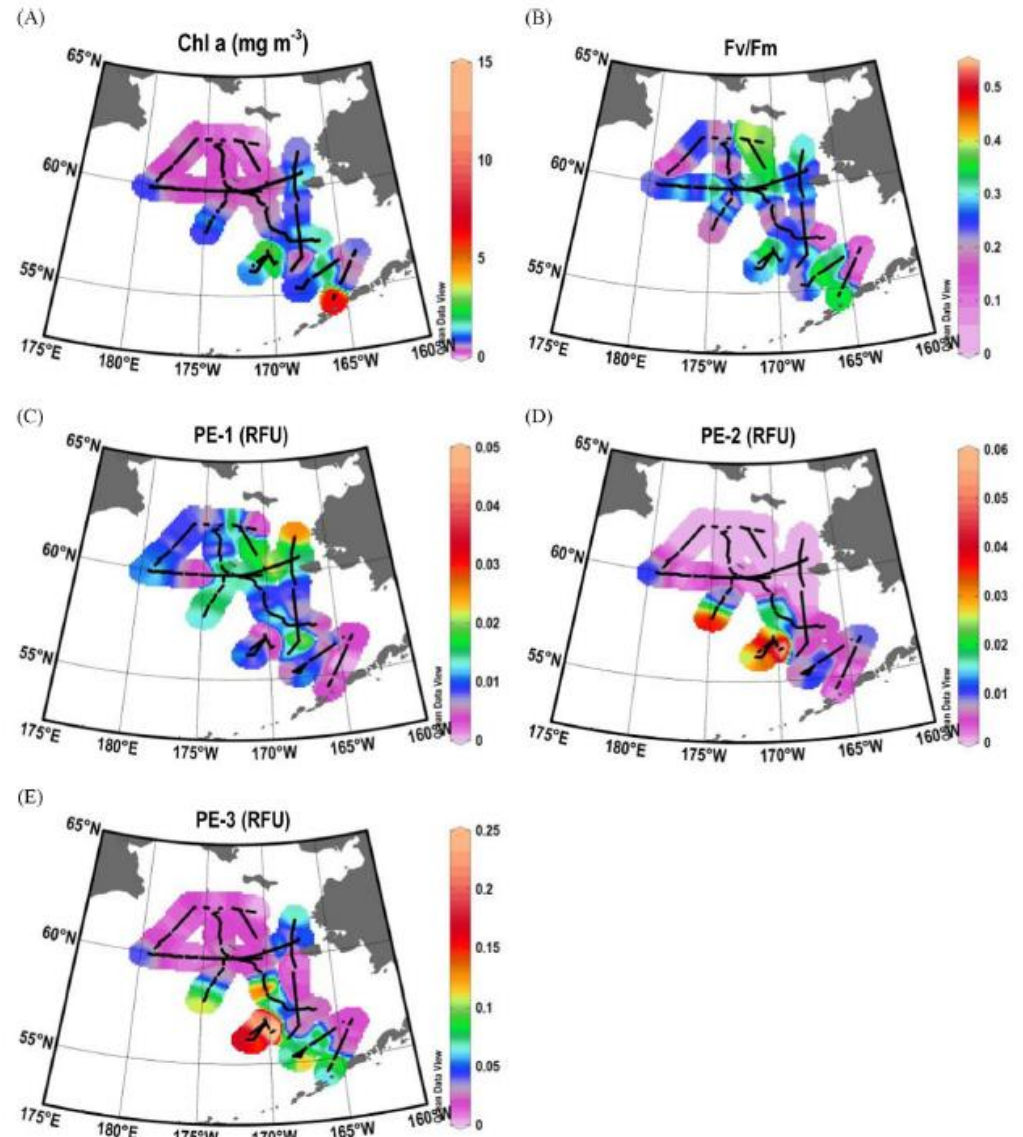
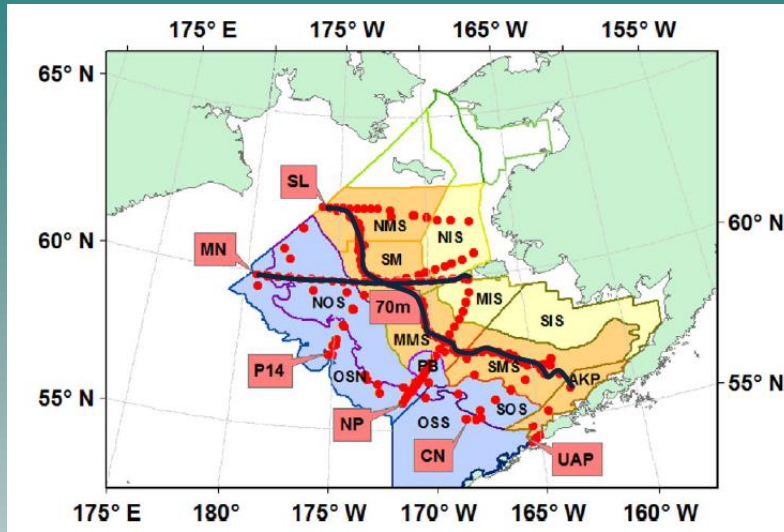
- 1:** A map of SS1 and SS2 ALF transect measurements and sample collection (CCE LTER Cruise P1106; June-July 2011). **2:** Relationship between **Chl concentration** ($\mu\text{g L}^{-1}$) and ALF measurements of **Chl fluorescence** normalized to Raman (CF/Rb) and variable fluorescence (Fv/Fm; 405 nm excitation). **3:** Relationship between **carbon biomass of autotrophic phytoplankton** (AC, $\mu\text{g C L}^{-1}$) and ALF measurements of **Chl fluorescence** normalized to Raman and variable fluorescence (Fv/Fm; 405 nm excitation). **4:** Relationship between **Synechococcus carbon biomass** (SYN; $\mu\text{g C L}^{-1}$) and ALF measurements of Raman-normalized **PE1 fluorescence** of oceanic cyanobacteria (PE1/Rg; 532 nm excitation).

ALF: California Current Synoptic Survey (SIO, NSF), CCE LTER, 2011



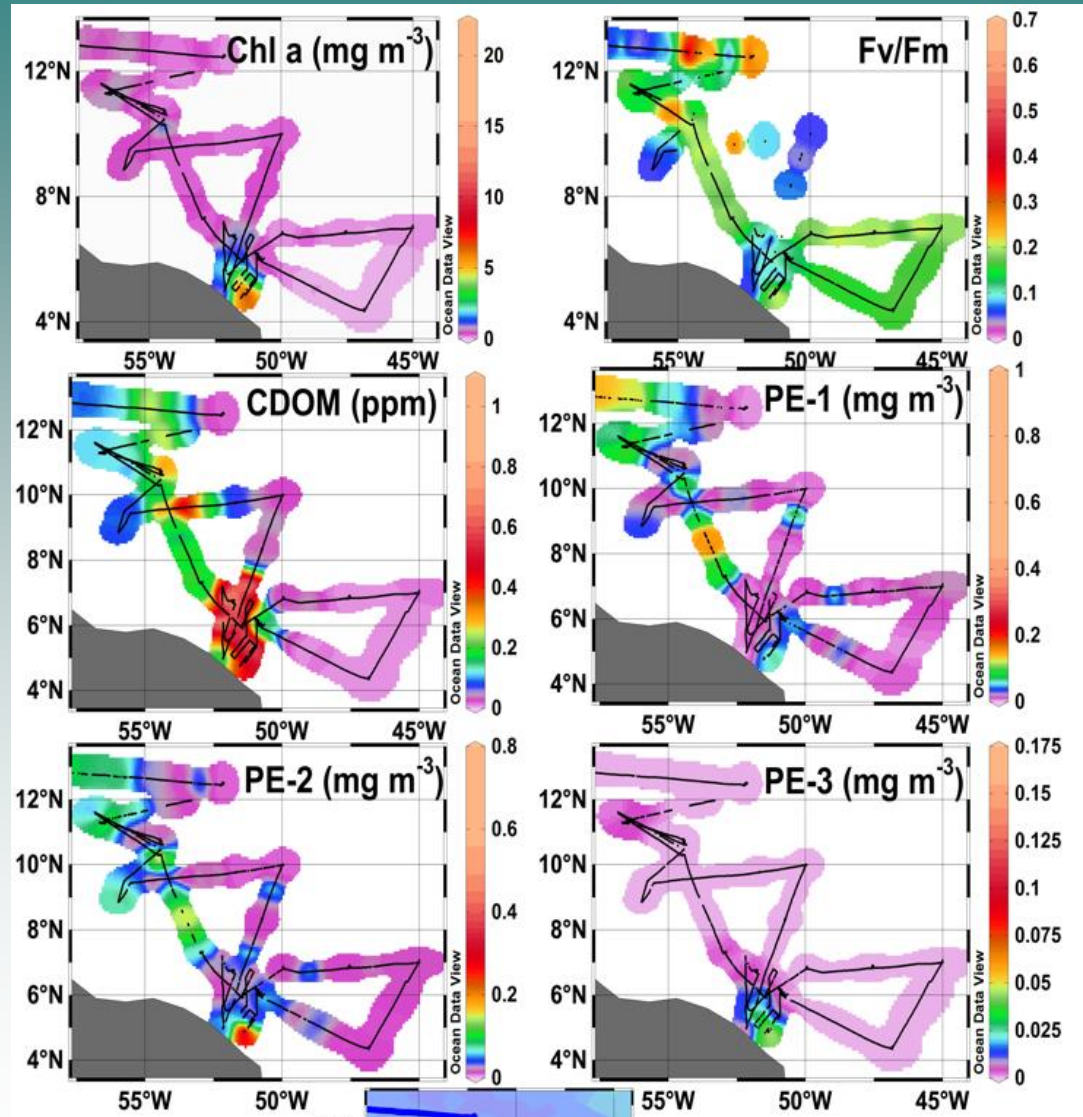
Transect distributions of Chl concentration (**Chl**, **3**), total phytoplankton carbon biomass (**AC**, **4**), *Synechococcus* carbon biomass (**SYN**, **5**), and fraction of *Synechococcus* in total phytoplankton carbon biomass (**SYN/AC**, **6**) were calculated from the ALF underway fluorescence measurements corrected for solar-induced non-photochemical quenching (NPQ). California Current Ecosystem (CCE, SIO) Long-Term Ecological Research (LTER, NSF) Process cruise P1106; 3-6 July 2011.

ALF: Phytoplankton characterization in Bering Sea



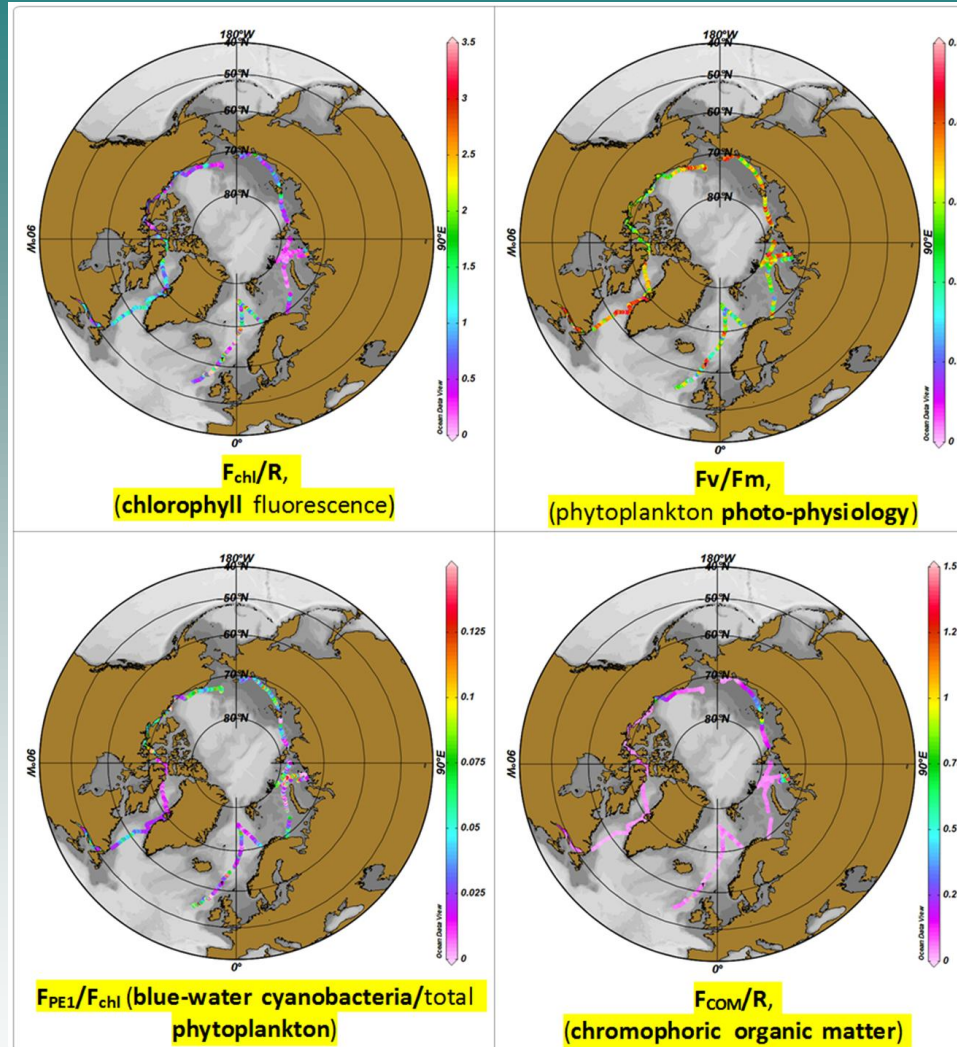
Goes, J.I., et al., Fluorescence, pigment and microscopic characterization of Bering Sea phytoplankton community structure and photosynthetic competency. *Deep-Sea Res. II* (2014), <http://dx.doi.org/10.1016/j.dsr2.2013.12.004>

ALF: Influence of Amazon River discharge on phytoplankton bio-geography



Goes, J.I., et al., Influence of the Amazon River discharge on the biogeography of phytoplankton communities in the western tropical north Atlantic. *Progress in Oceanography* 120, 29-40 (2014).

ALFA: Arctic Seas survey (8 month deployment)



Global-scale underway ALFA measurements to survey the impact of climate change on bio-environmental situation in the Arctic Seas (May – Dec 2013, R/V Tara; Dr. Boss (U. of Maine)).

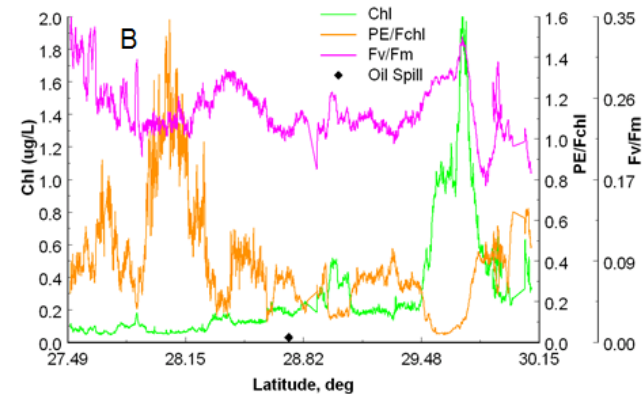
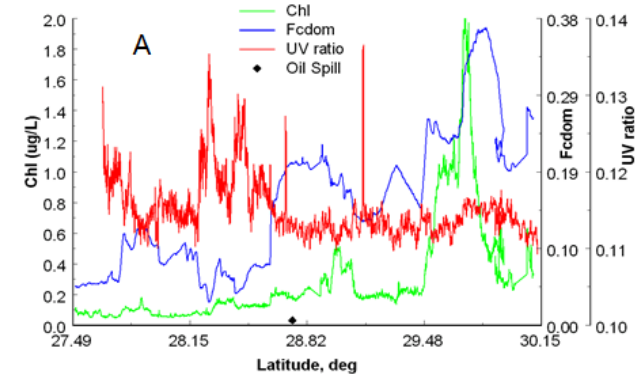
Matsuoka, A., et al. Pan-Arctic optical characteristics of colored dissolved organic matter: Tracing dissolved organic carbon in changing Arctic waters using satellite ocean color data. *Remote Sensing of Environment*, V. 200, pp. 89-101; <https://doi.org/10.1016/j.rse.2017.08.009> (2017)

ALF: Evaluation of bio-environmental impacts of Deepwater Horizon Oil Spill (RAPID/NSF 2010)

PIs: A. Chekalyuk, A. Subramaniam and A. Thurnherr (LDEO of Columbia University)

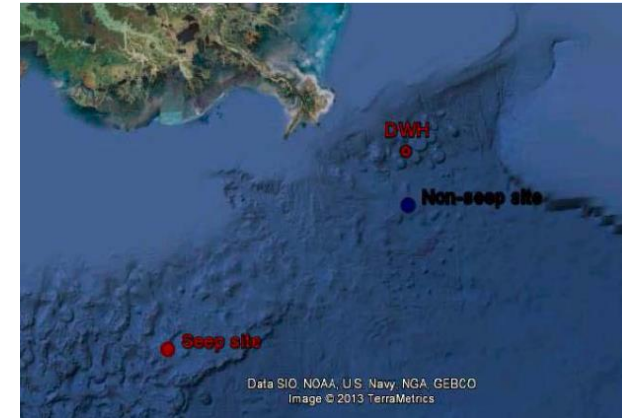
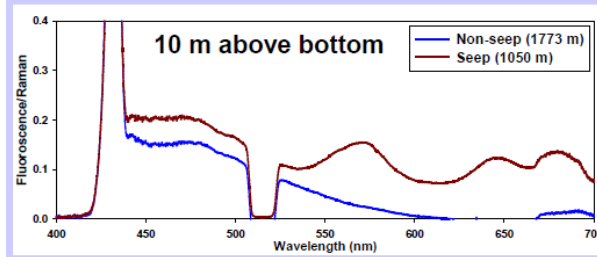
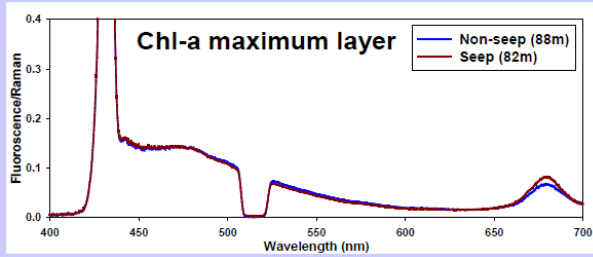
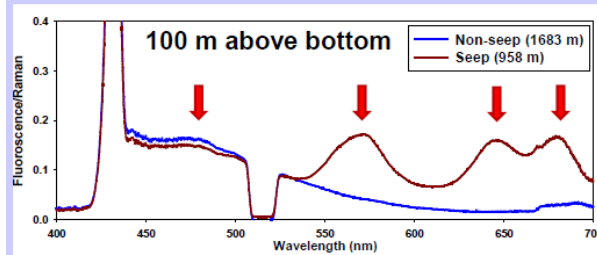
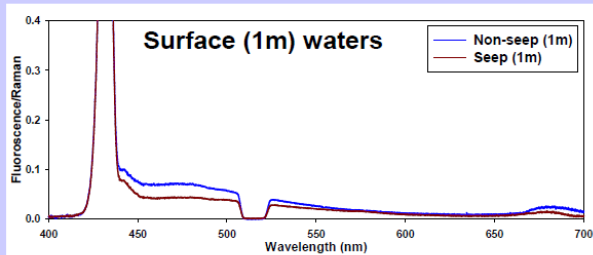
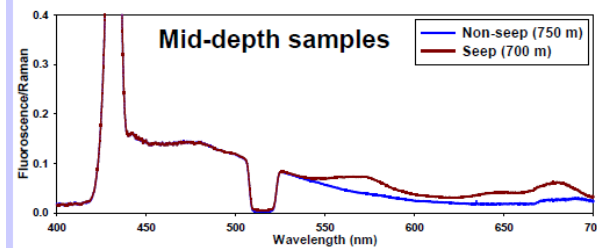
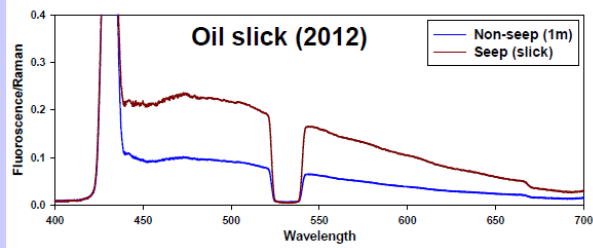


Fig. 1 A map of the major underway transect measurements and sampling stations in the area of study. Location of the Deepwater Horizon accident is marked with the flag.



Horizontal distributions of the key fluorescence variables retrieved from the ALF underway transect measurements in the Gulf of Mexico (a synoptic-scale transect is displayed with a red line in Fig. 1). The black diamond indicates a location of the Deepwater Horizon accident.

ALF: Unique Spectral Signatures Near Oil Seeps



The ALFA can be used to rapidly characterize fluorescent materials suspended in natural waters including phytoplankton pigments and fresh oil.

Note: The **unique fluorescence signatures** near oil seeps *could not be detected by conventional Chl or oil/hydrocarbon fluorometers.*

Fluorescence spectra of water samples near the oil seep (brown graphs) vs. water spectra at non-seep location.

Note: The deep **sample spectra** at the seep site *did not resemble* **fresh oil or PAHs.**

ALF: Validation of Satellite Chl data in Delaware Bay



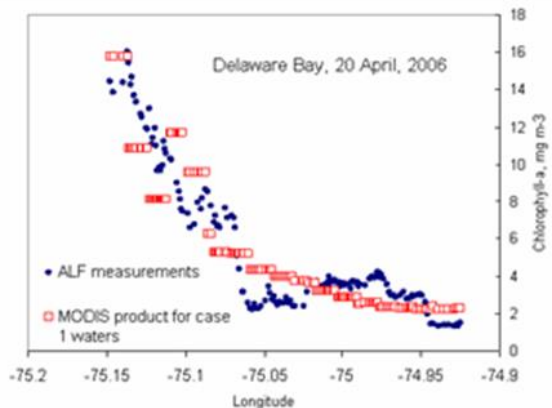
A



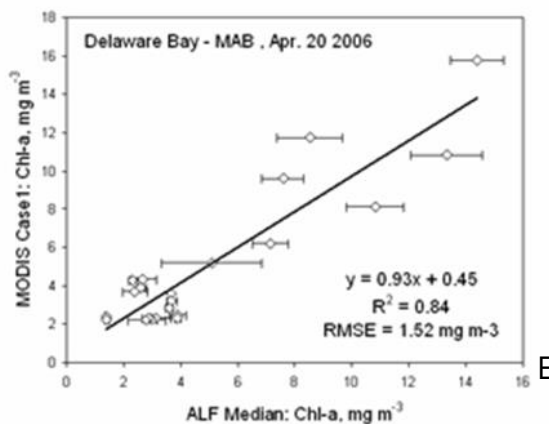
B



C



D



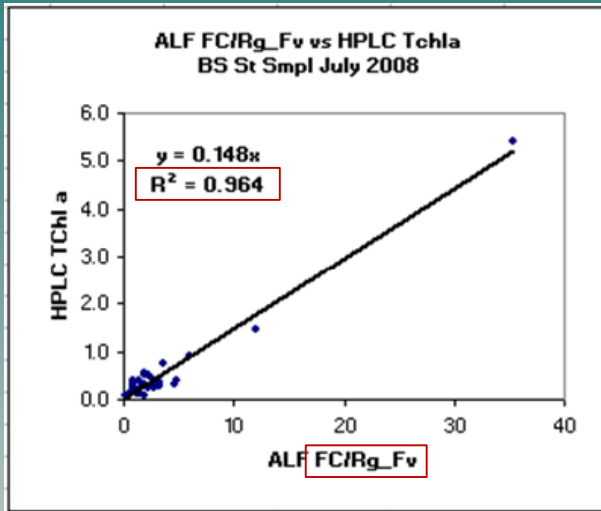
E

A,B: High-resolution ALF underway measurements on a speed boat (NASA – U. of Delaware; 2006).

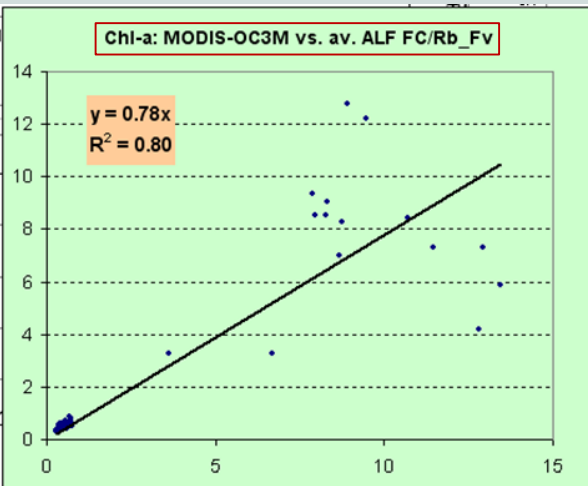
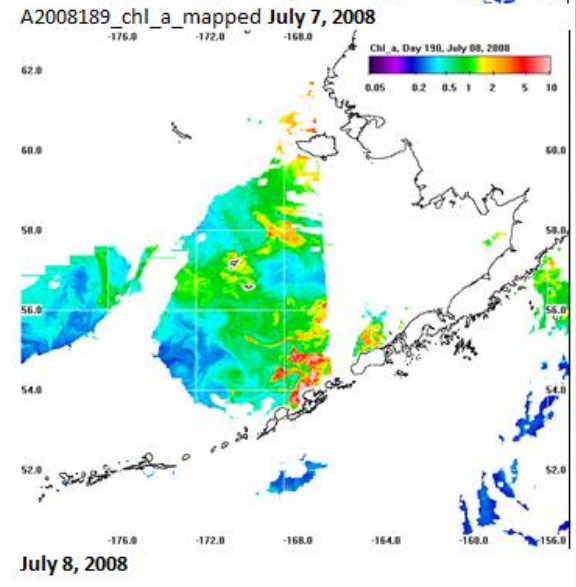
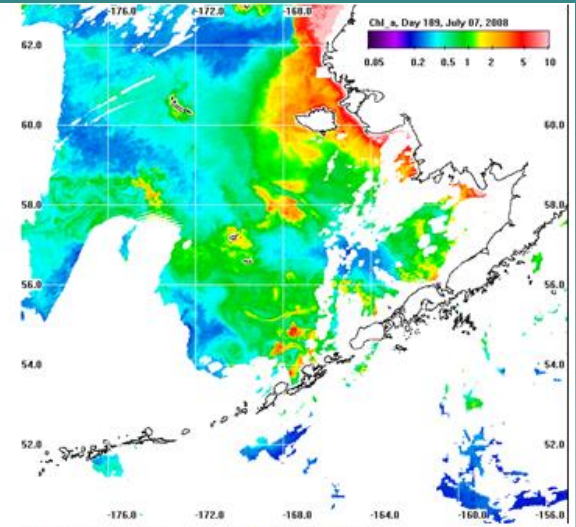
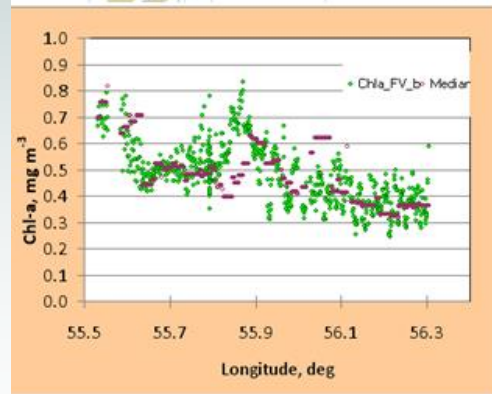
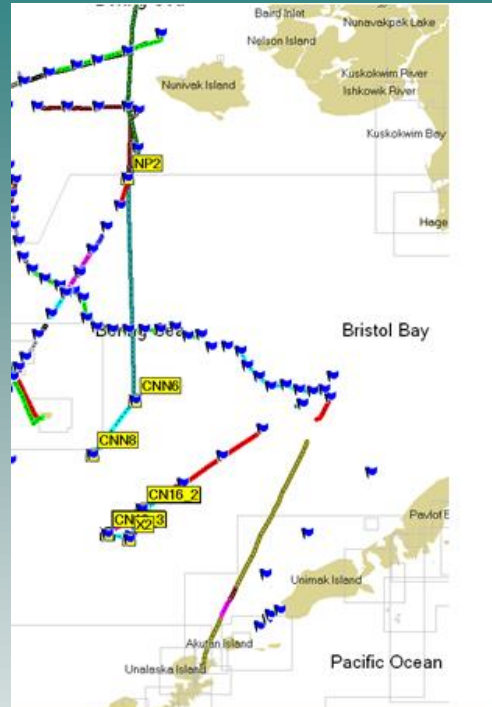
C: A map of the ALF transect survey in the Delaware Bay mouth.

D, E: ALF Chl measurements vs. satellite ocean color data.

ALF: Validation of Satellite Chl data (Bering Sea, 2008)

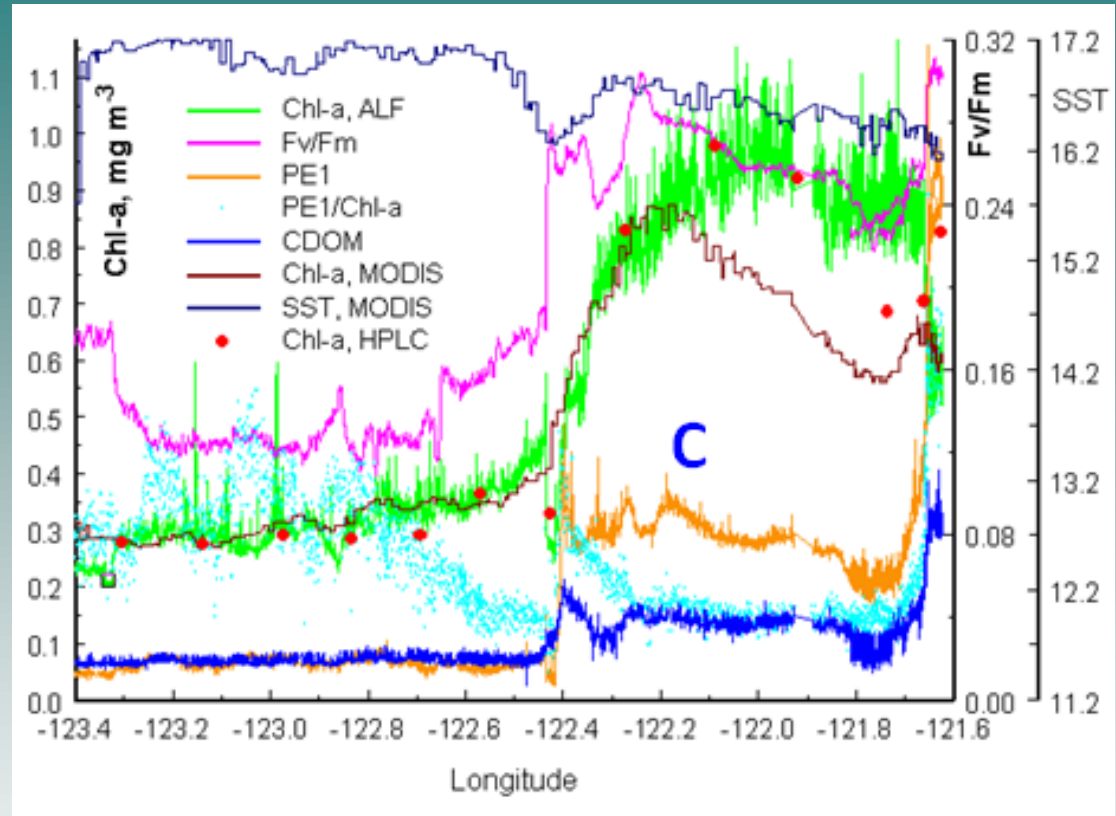
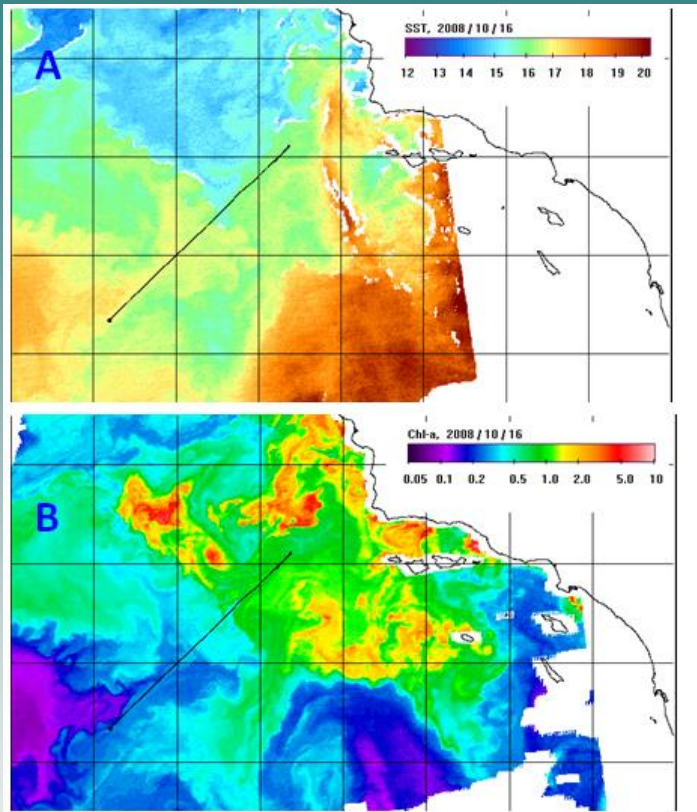


Calibration of the ALF Chl fluorescence corrected for the solar-induced NPQ quenching, using HPLC Chl measurements



Matching MODIS Aqua Chl and ALF underway Chl data (ALF measurements by Dr. Goes (LDEO))

ALF: Validation of Satellite Chl Data in California Current



Matching MODIS Aqua SST (A, C) and Chl-a (B, C) data with ALF shipboard underway measurements and HPLC Chl-a sampling (C).

ALF measurements can be used:

- for **validation** of satellite algorithms and assessment of uncertainties;
- to support development of the **new ocean color products** (e.g. phycobiliprotein biomass, phytoplankton functional types, etc.).

ALF: References

1. Chekalyuk, A.M. and M. Hafez. Advanced laser fluorometry of natural aquatic environments. *Limnol. Oceanogr. Methods*, 6:591-609 (2008)
2. Chekalyuk, A. and M. Hafez. Next Generation Advanced Laser Fluorometry for Characterization of Natural Aquatic Environments: New Instruments. *Optics Express*. 21, 14181-14201 (2013)
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8. Goes, J. I., et al. Fluorescence, pigment and microscopic characterization of Bering Sea phytoplankton community structure and photosynthetic competency in the presence of a Cold Pool during summer. *Deep-Sea Research –II* (2013) doi:10.1016/j.dsr2.2013.12.004
9. Chekalyuk, A.M., et al. The Aquatic Laser Fluorescence Analyzer: field evaluation in the northern Gulf of Mexico. *Optics Express*. 22, 21641-21656 (2014)
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12. Matsuoka, A., et al. Pan-Arctic optical characteristics of colored dissolved organic matter: Tracing dissolved organic carbon in changing Arctic waters using satellite ocean color data. *Remote Sensing of Environment*, V. 200, pp. 89-101; <https://doi.org/10.1016/j.rse.2017.08.009> (2017)

Patents:

- PT1. Chekalyuk, A.M. “Spectral and Temporal Laser Fluorescence Analysis such as for Natural Aquatic Environments”, US Patent 8970841 (Mar. 3, 2015).
- PT2. Chekalyuk, A.M. “Optical Analysis of Emissions from Stimulated Liquids”, US Patent 9618449 (Apr 11, 2017).

Conclusions:

- **Aquatic Laser Fluorescence (ALF)** analysis can be used in a broad range of applications, including analysis of **phytoplankton pigments, photo-physiology, community structure, CDOM, oil and PAHs**
- Combining **spectral** and **temporal** measurements of **laser-stimulated emission** provides rich information for scientific and environmental studies
- The **ALF technique can serve as a useful tool** for oceanographic research, environmental monitoring, biological, industrial applications, health care, etc.