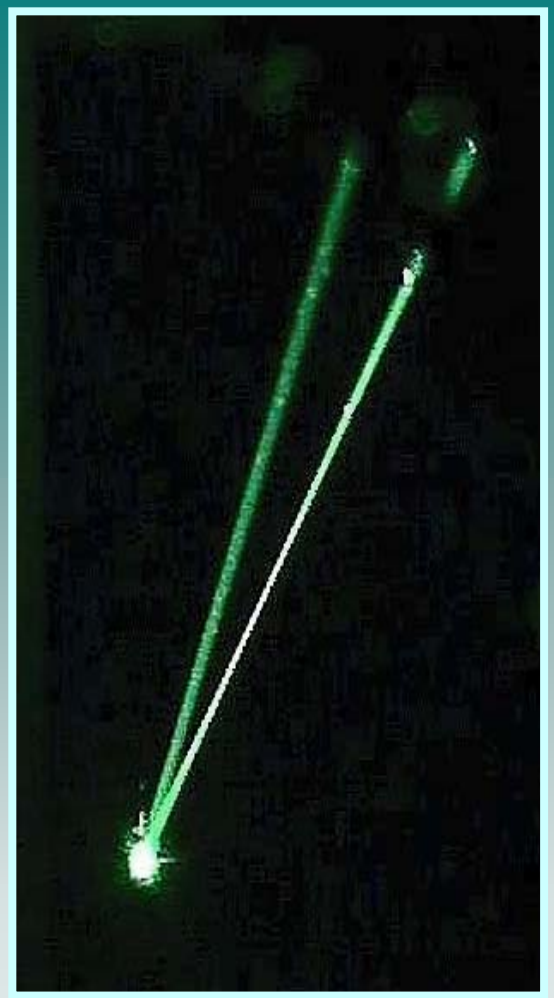
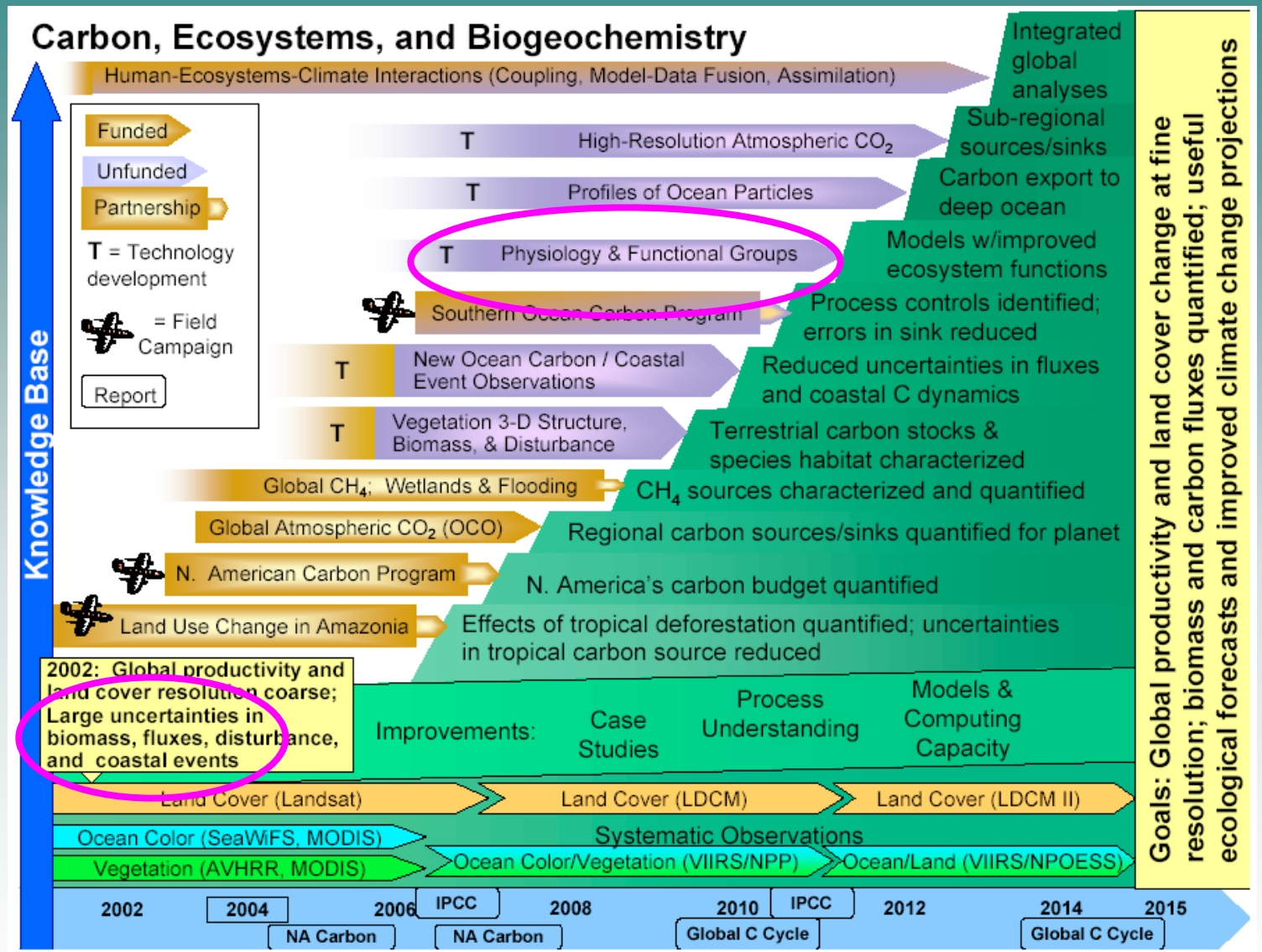


Laser Fluorescence Analysis of Phytoplankton: Beyond Chlorophyll Concentration

A.M. Chekalyuk[1,3], F.E. Hoge[3],
R.N. Swift[2] , J.K. Yungel[2], P.E. Lyon[2]

[1]Hampton University, [2]EG&G Services,
[3]NASA Goddard Space Flight Center
Wallops Flight Facility, chekaluk@osb.wff.nasa.gov

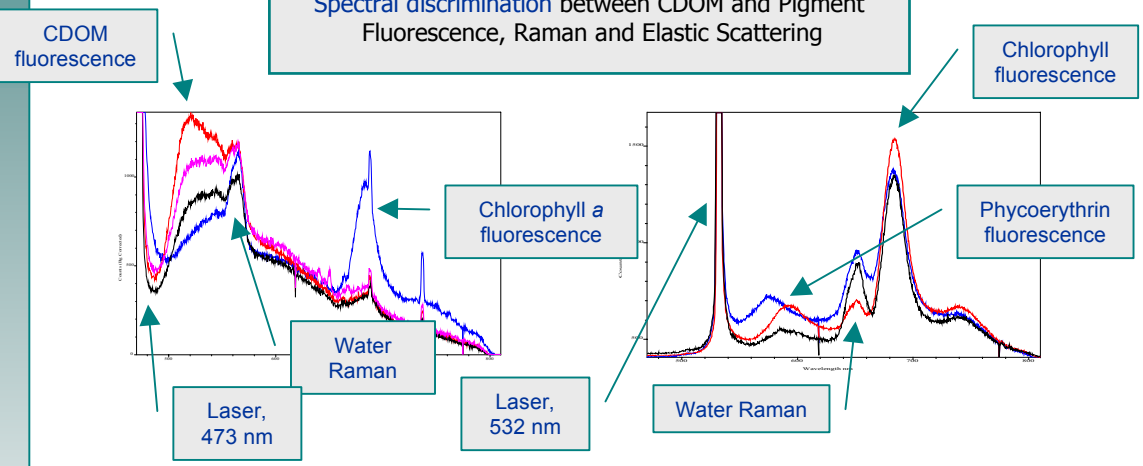
Objective: Remote Assessment of Phytoplankton Physiology, Pigments and Functional Groups



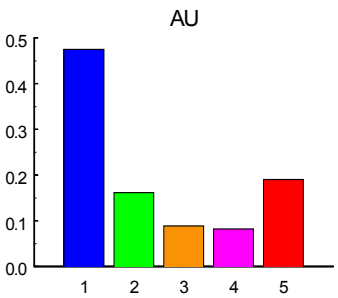
Active Fluorescence: A powerful Analytical Tool

Optical Complexity of Coastal Waters: CDOM, complex algal composition, suspended sediments, high turbidity

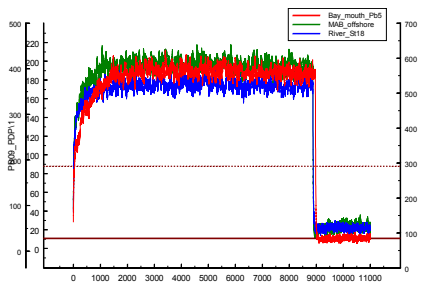
Spectral discrimination between CDOM and Pigment Fluorescence, Raman and Elastic Scattering



Selective spectral fluorescence excitation to assess phytoplankton pigment and taxonomic composition



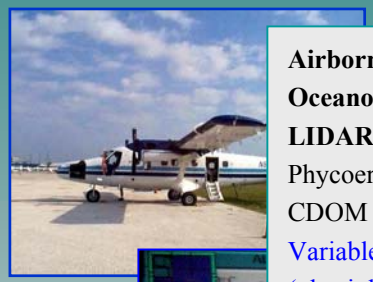
Fluorescence induction assessment of phytoplankton physiology



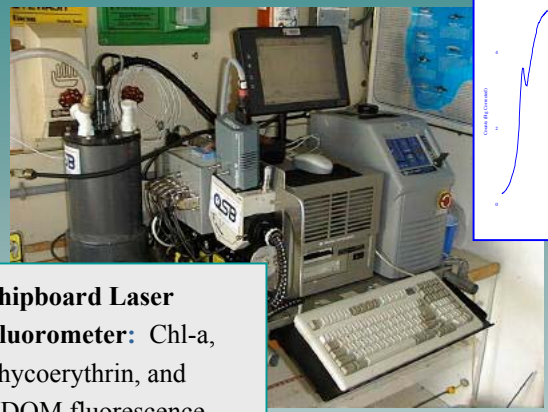
Active Fluorescence:

- **High sensitivity & Spectral discrimination** between CDOM and pigment fluorescence, Raman and elastic scattering
- **Enhanced selectivity** provided by spectral fluorescence excitation
- Potential for **assessment of algal pigments, physiology, and dominant groups**
- **Variety of platforms:** ships, drifters, buoys, autonomous vehicles, airplanes (LIDAR), satellites (PhyLM?)
- New: **Works remotely** and can be **as accurate as HPLC**

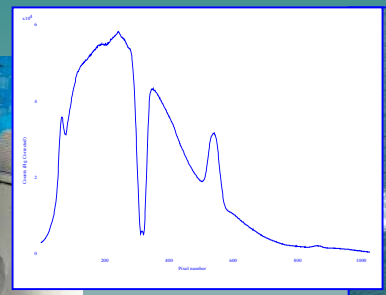
October 2003: Airborne/Shipboard Laser Measurements in the Chesapeake and Delaware Bays

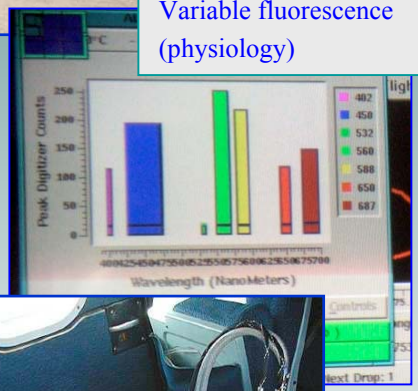


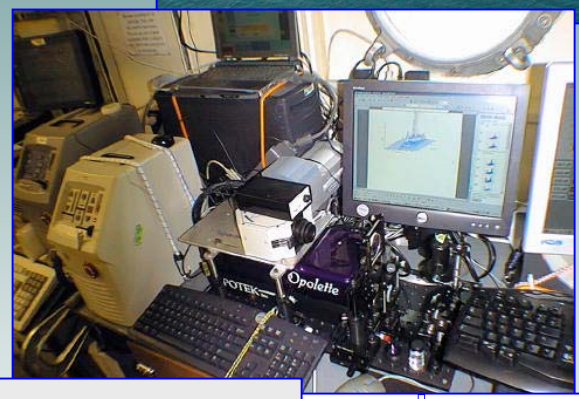
Airborne Oceanographic LIDAR: Chl-a, Phycoerythrin, and CDOM fluorescence; Variable fluorescence (physiology)

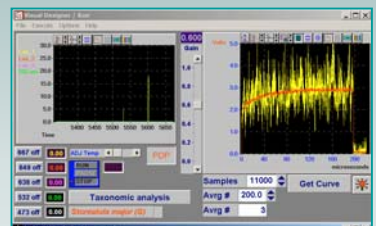


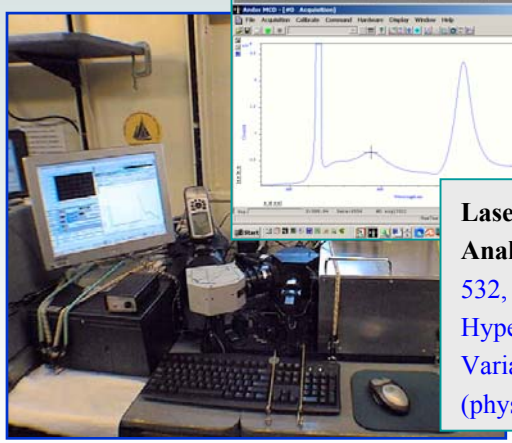
Shipboard Laser Fluorometer: Chl-a, Phycoerythrin, and CDOM fluorescence





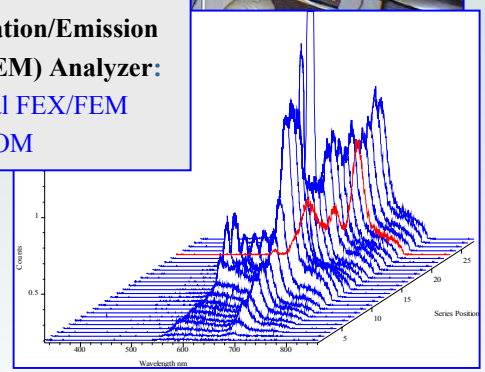


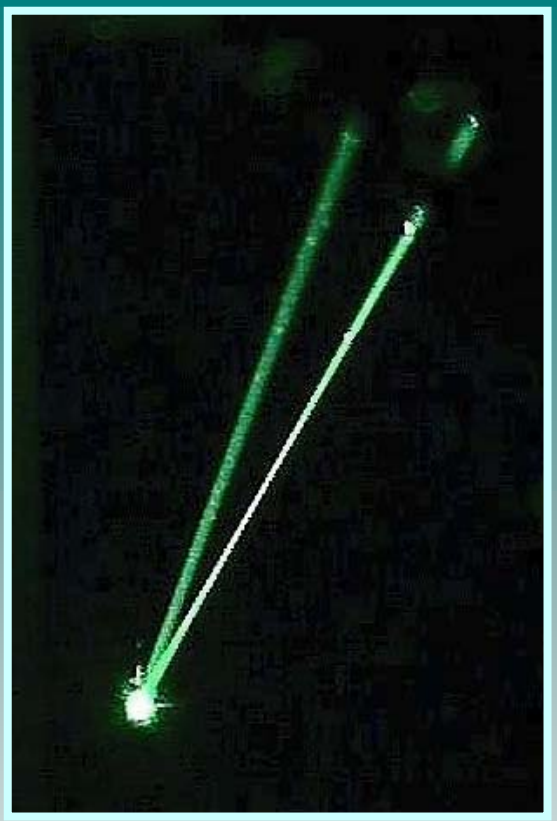




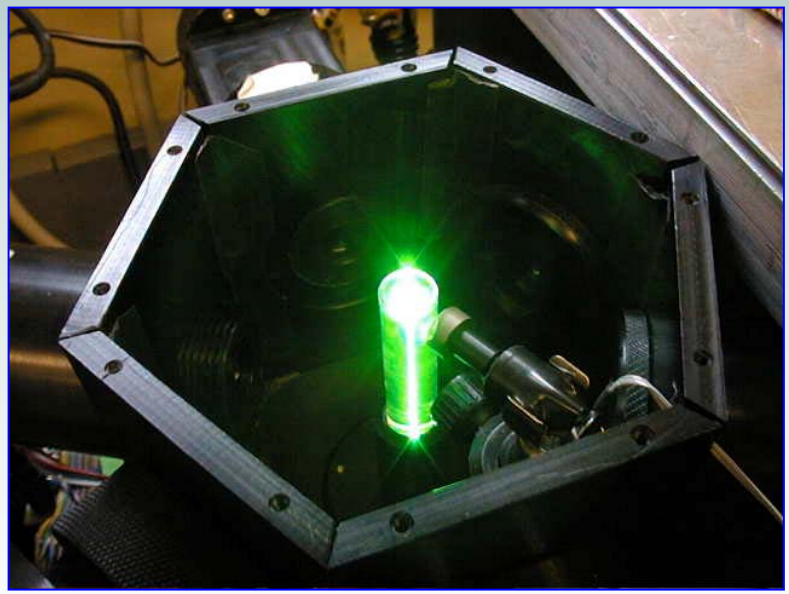
Laser Phytoplankton Analyzer: FEX_5 (473, 532, 639, 651, 666 nm), Hyperspectral FEM, Variable fluorescence (physiology)

Laser Excitation/Emission Matrix (LEEM) Analyzer: Hyperspectral FEX/FEM pigment/CDOM

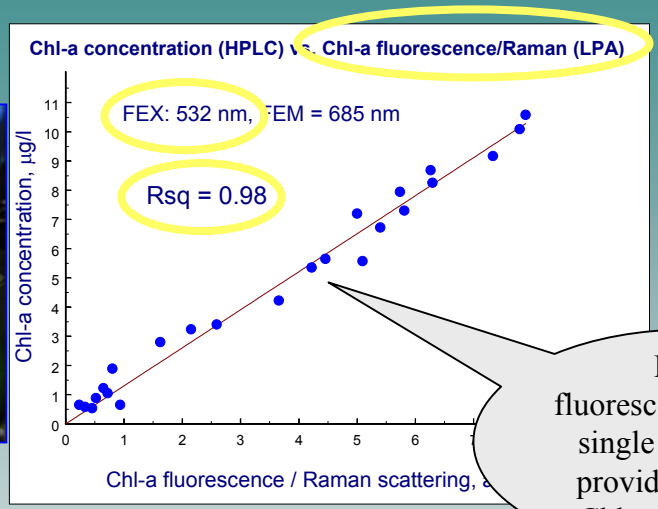




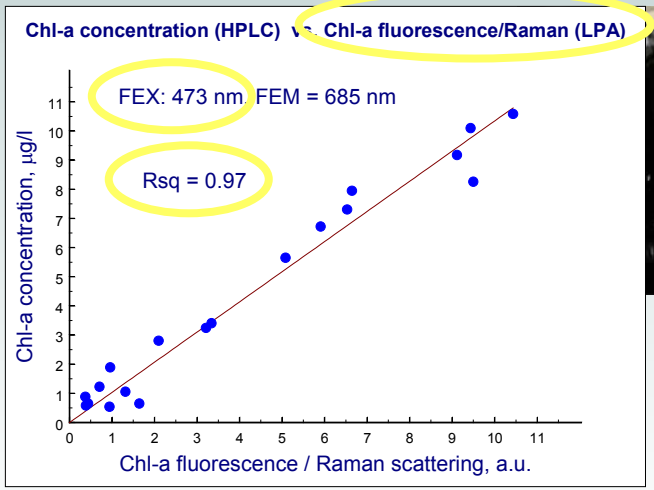
1. Laser Fluorescence EMission (FEM) Assessment of Chlorophyll-a Concentration

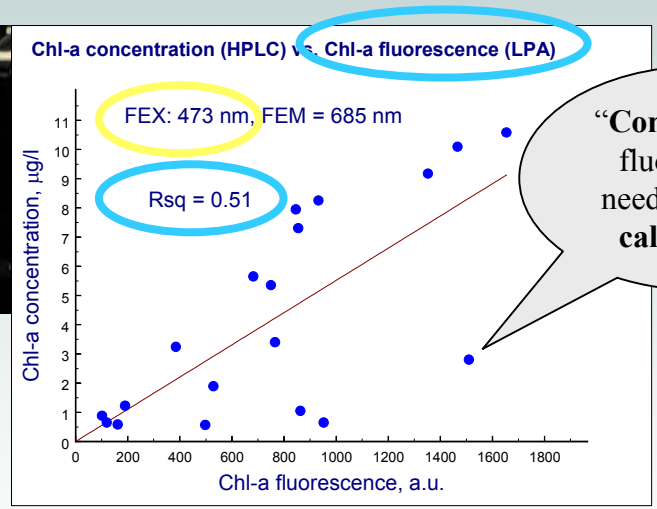


LPA Field Test: Laser FEM Assessment of Chl-a Concentration Can be as Precise, as HPLC Analysis!



Laser fluorescence/Raman: single calibration provides accurate Chl-a assessment

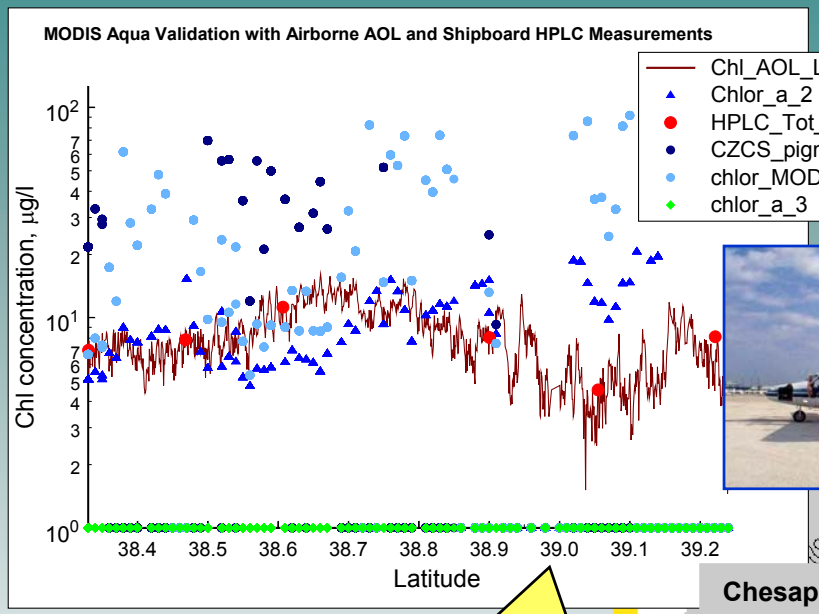


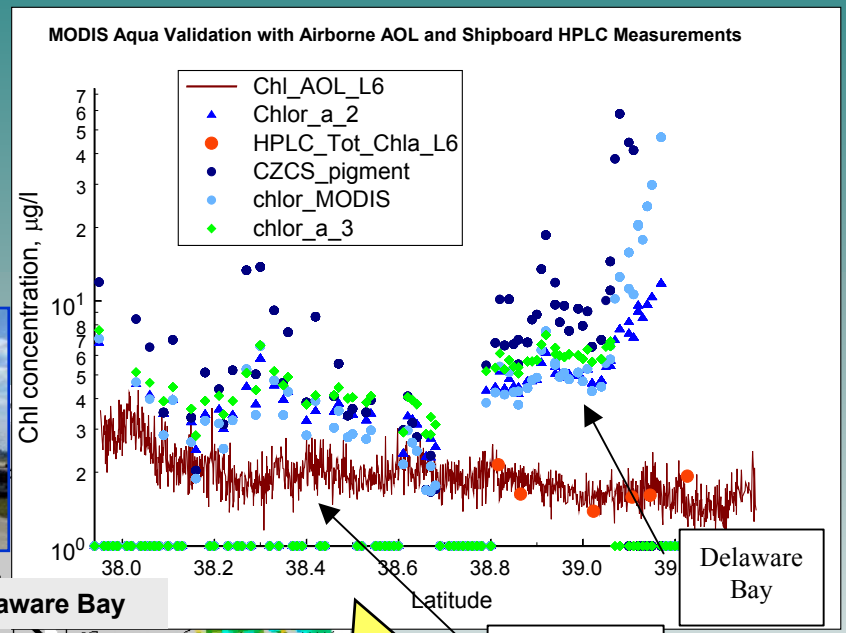


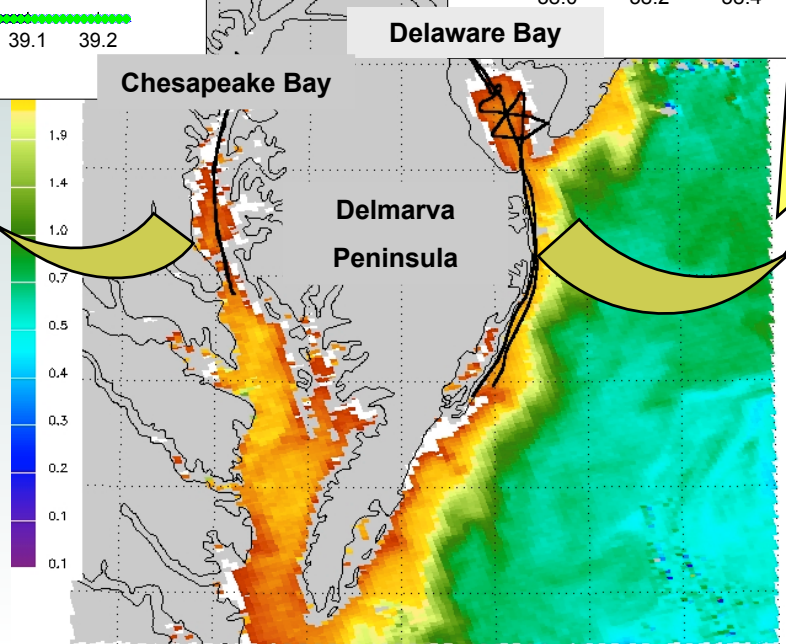
"**Conventional**" fluorometry: needs **frequent calibrations**

Airborne LIDAR + Shipboard HPLC: Validation of Chl-a Ocean Color Algorithms in Coastal and Estuarine Areas

Satellite-Airborne-Shipboard Experiment, October 2003



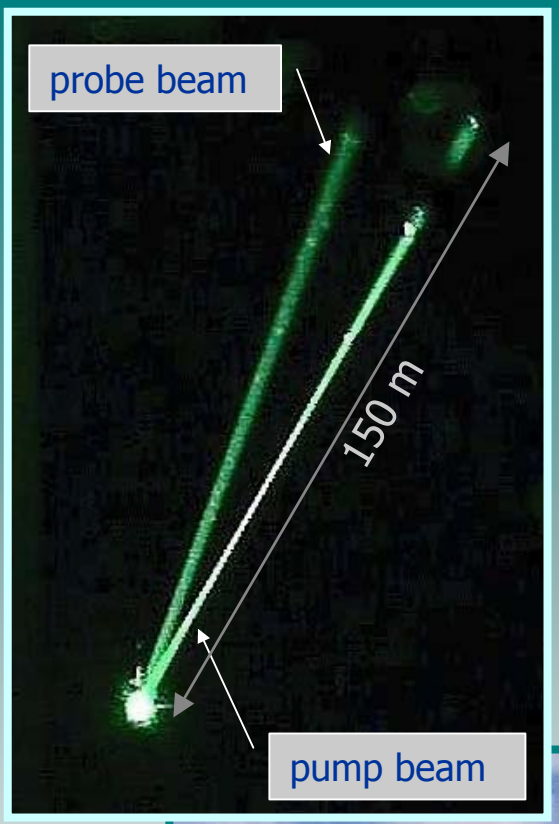


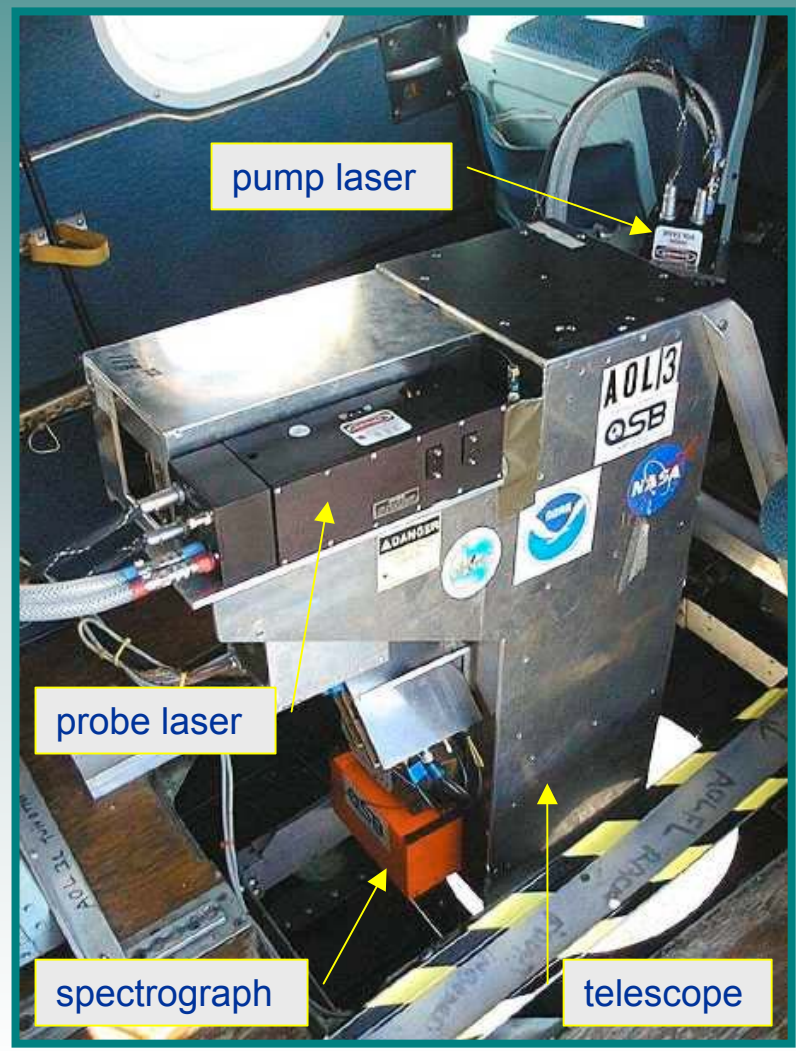


- **Shipboard HPLC**: Conversion LIDAR Chl fluorescence into Chl concentration, µg/l
- **Airborne LIDAR**: filling space/time satellite-ship gap
- **Satellite Chl algorithm validation in the Chesapeake and Delaware Bays**: "Chl-a2" worked best of all

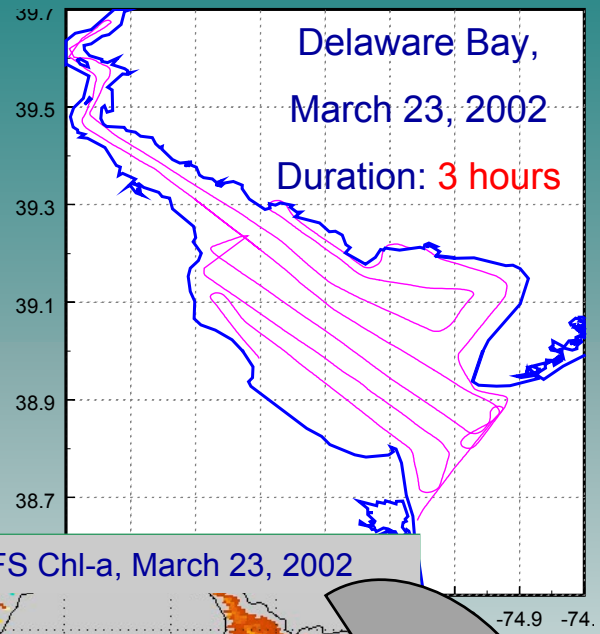
- Pump&Probe (P&P) LIDAR: Airborne Fluorescence Assessment of Phytoplankton Physiology

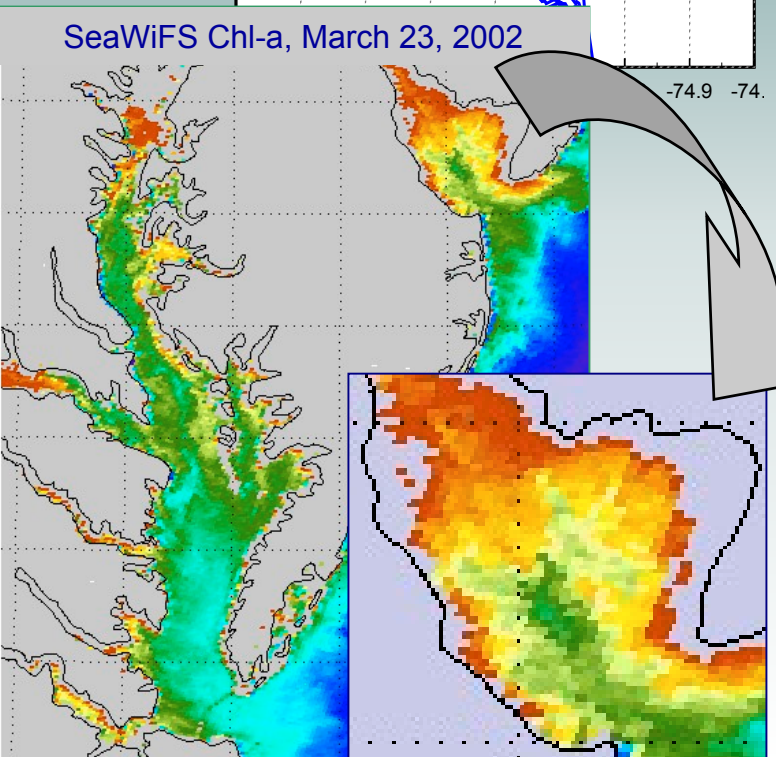
P&P LIDAR: An Upgrade of the Airborne Oceanographic LIDAR (AOL)

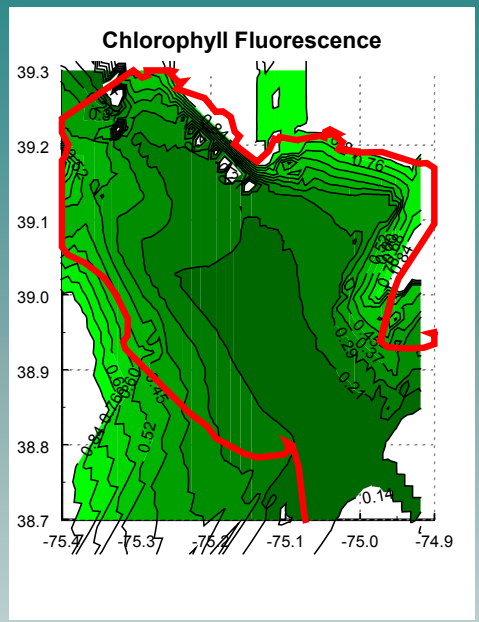


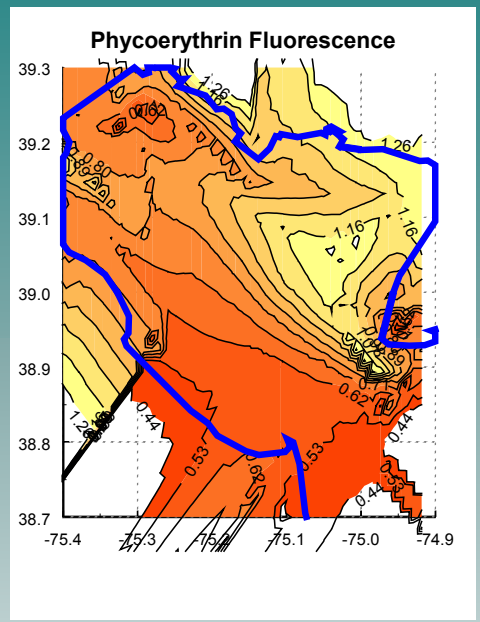


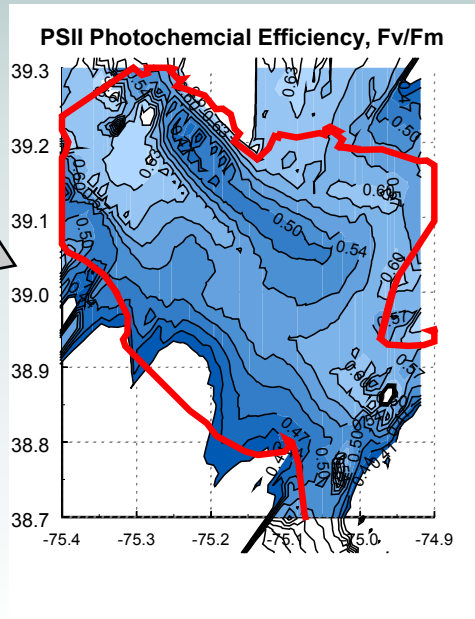
P&P LIDAR: 2D Mapping

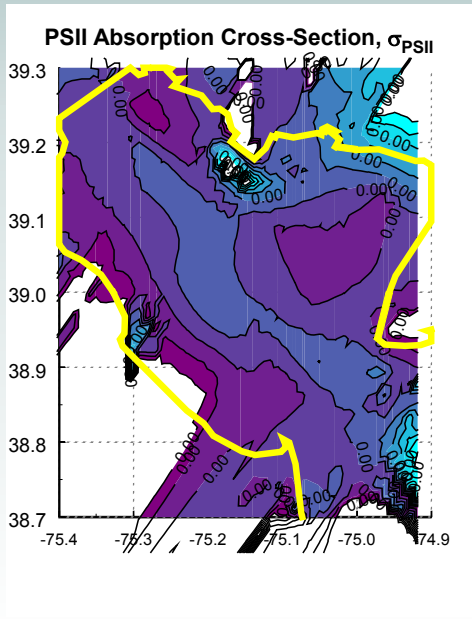




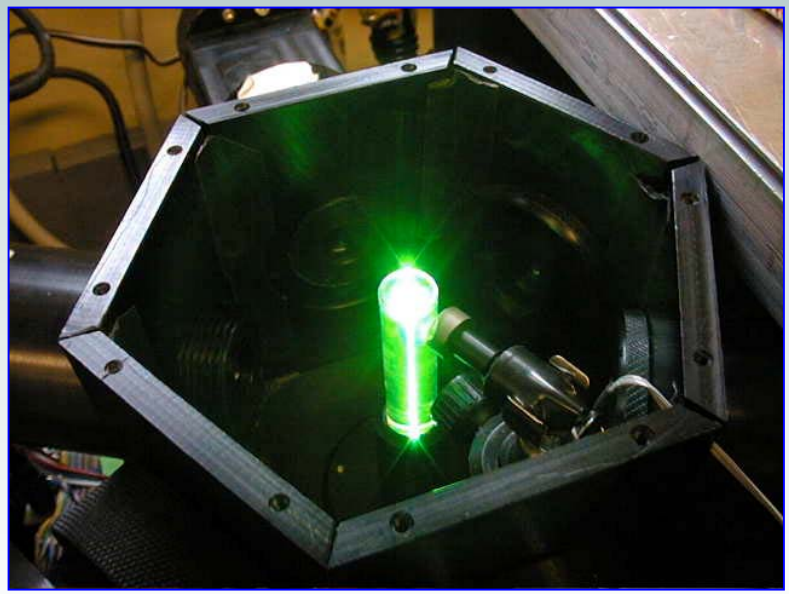




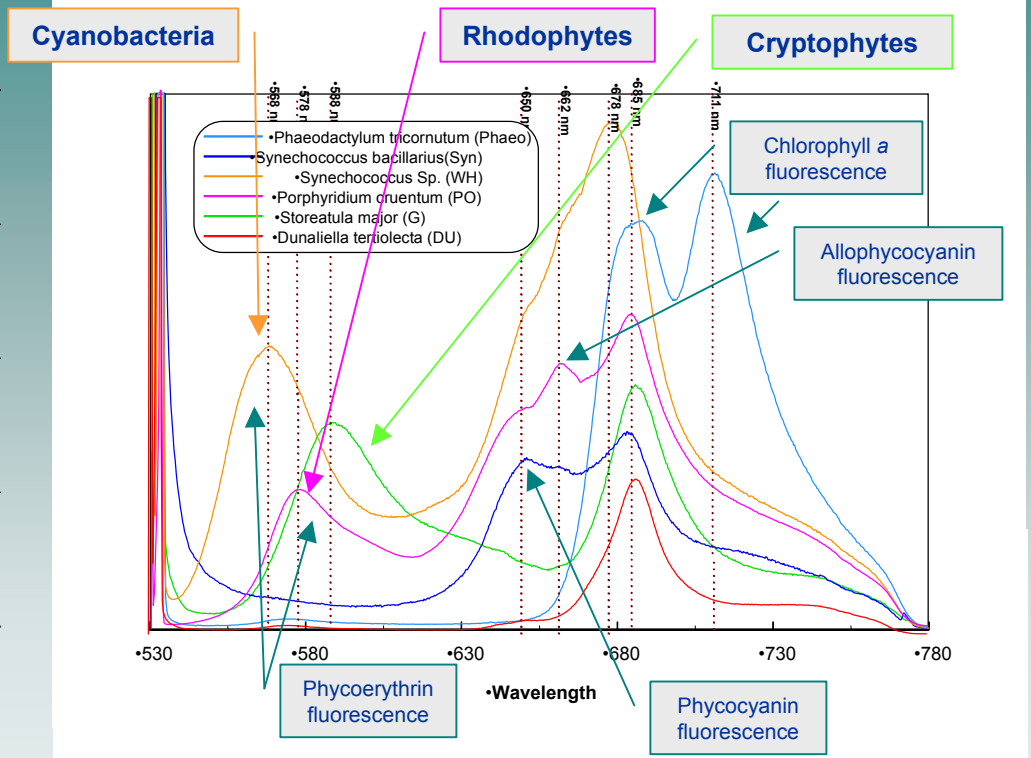




3. Laser Fluorescence EXcitation (FEX) / EMission (FEM) Analysis: Improved Phytoplankton Pigment/Group Characterization



LPA: Spectral diversity of emission bands allows improved algal/pigment characterization

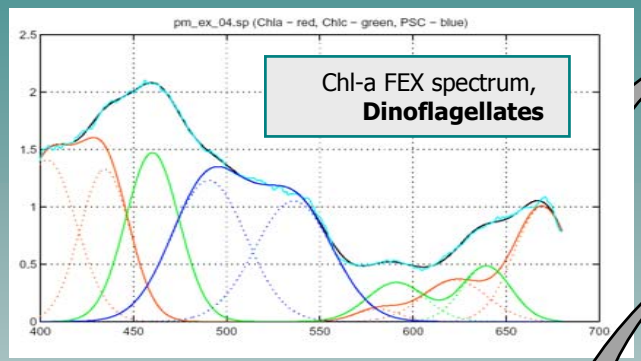


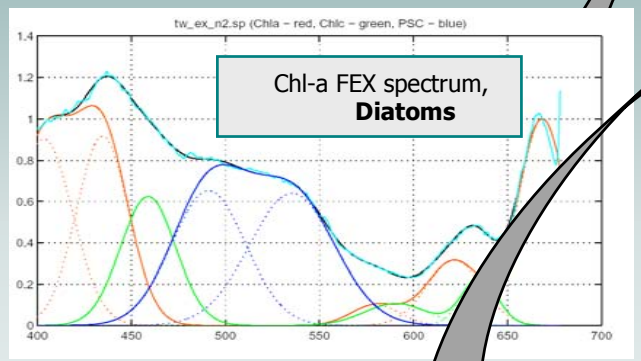
Fluorescence EMission (FEM) spectra of phycobilin-containing phytoplankton and cyanobacteria. Chl-a FEM signatures of chlorophyte *Dunaliella tertiolecta* and diatom *Phaeodactylum tricornutum* are displayed for comparison

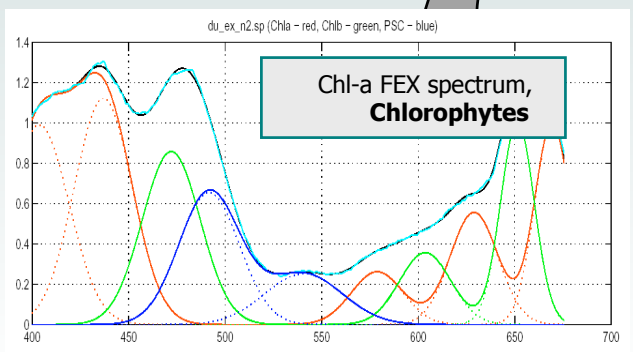
Table 1. Strains of phytoplankton studied with the LPA system

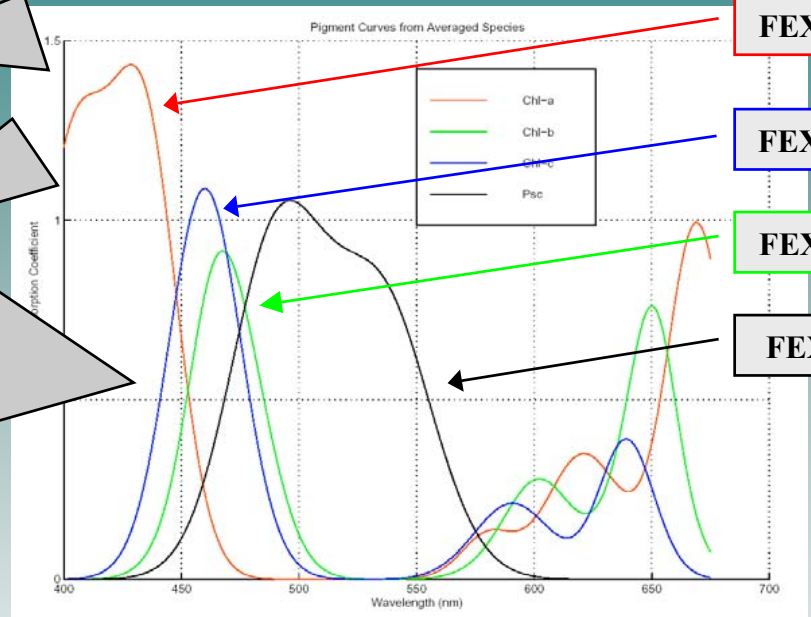
Dunaliella tertiolecta	Chlorophyte
Nanochloris atomus	Chlorophyte
Storeatula major	Cryptophyte
Synechococcus bacillarius	Cyanobacteria
Synechococcus sp.	Cyanobacteria
Phaeodactylum tricornutum	Diatom
Skeletonema costatum	Diatom
Thalassiosira weissflogii	Diatom
Navicula sp.	Diatom
Aureococcus anophagefferens	Pelagophyte
Pycnococcus provalosii	Prasinophyte
Pavlova lutheri	Prymnesiophyte
Prorocentrum minimum	Dinoflagellate
Porphyridium cruentum	Rhodophyte

FEX Spectra of NPB Algal Species: Assessment of Pigment-Specific FEX Components





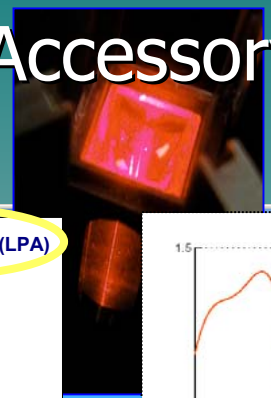


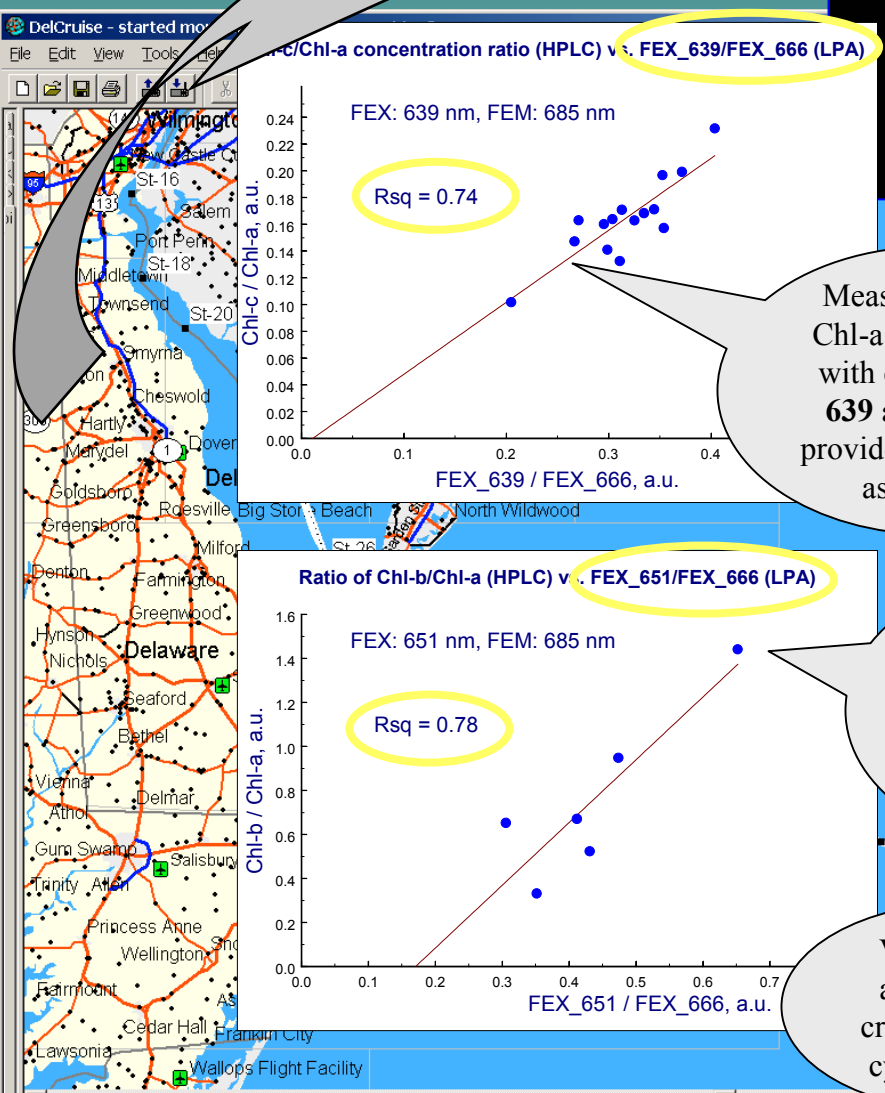


Pigment-specific FEX components, FEX_{Chl-a}, FEX_{Chl-b}, FEX_{Chl-c} and FEX_{PSC}:

- Deconvolution of FEX species spectra into 10 gaussian components (best fitting)
- Reconstruction of pigment-specific FEX components for each species
- Averaging of the pigment-specific FEX components over a group of species examined

LPA Field Test: Laser Fluorescence Assessment of None-Fluorescent Accessory Pigments

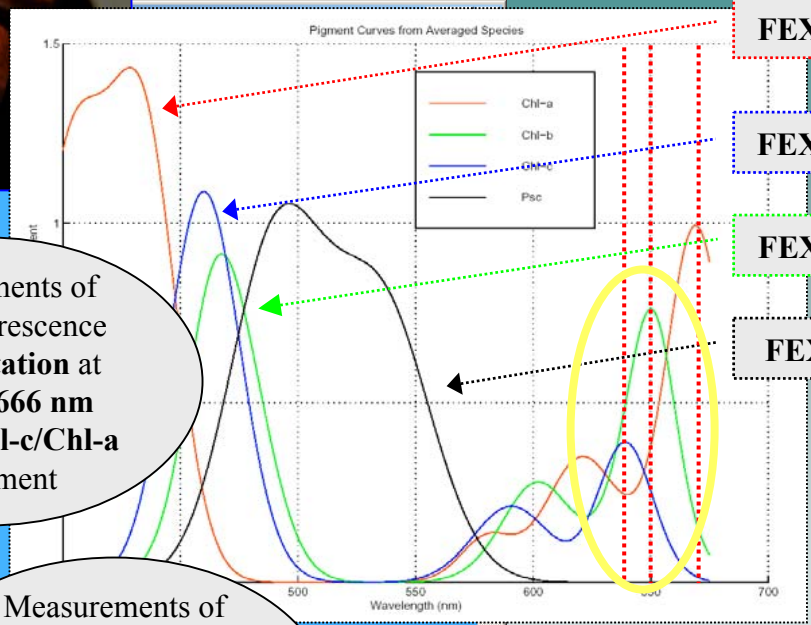


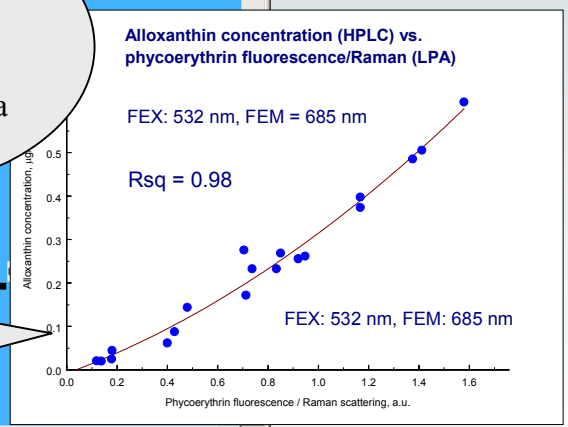


Measurements of Chl-a fluorescence with **excitation** at **639 and 666 nm** provide **Chl-c/Chl-a** assessment

Measurements of Chl-a fluorescence with **excitation** at **651 and 666 nm** provide Chl-b/Chl-a assessment

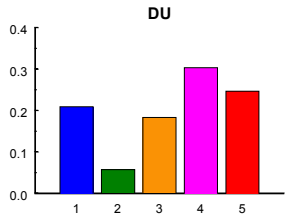
Variability in abundance of cryptophytes vs. cyanobacteria?



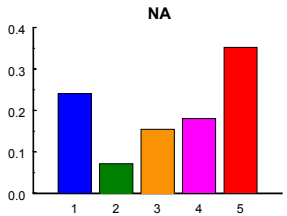


Laser Pigment Analyzer: Identification of Major Algal Groups with 5-wavelength FEX of Chl-a

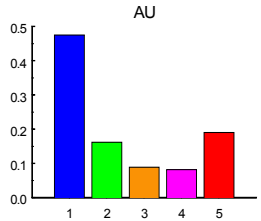
Dunaliella Tertiolecta
Chlorophyte



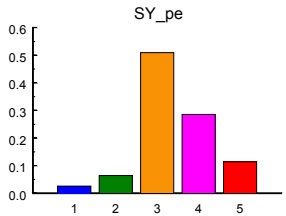
Nanochloris atomus
Chlorophyte



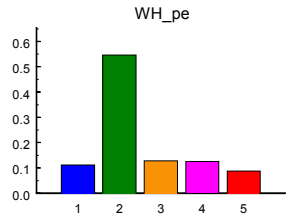
Aureococcus anophagefferens
Pelagophyte

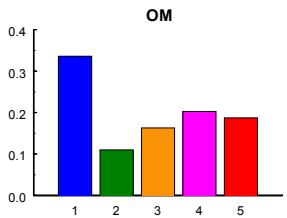


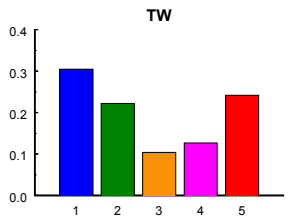
Synechococcus bacillarius
Cyanobacteria

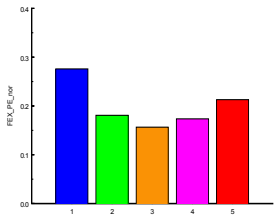


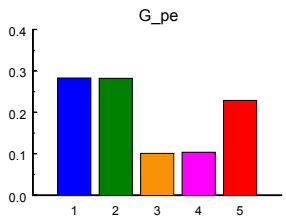
Synechococcus sp.
Cyanobacteria

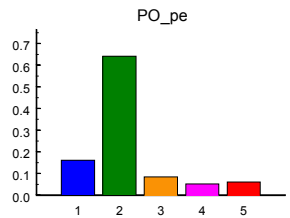












Pycnococcus provalosii
Prasinophyte

Thalassiosira weissflogii
Diatom

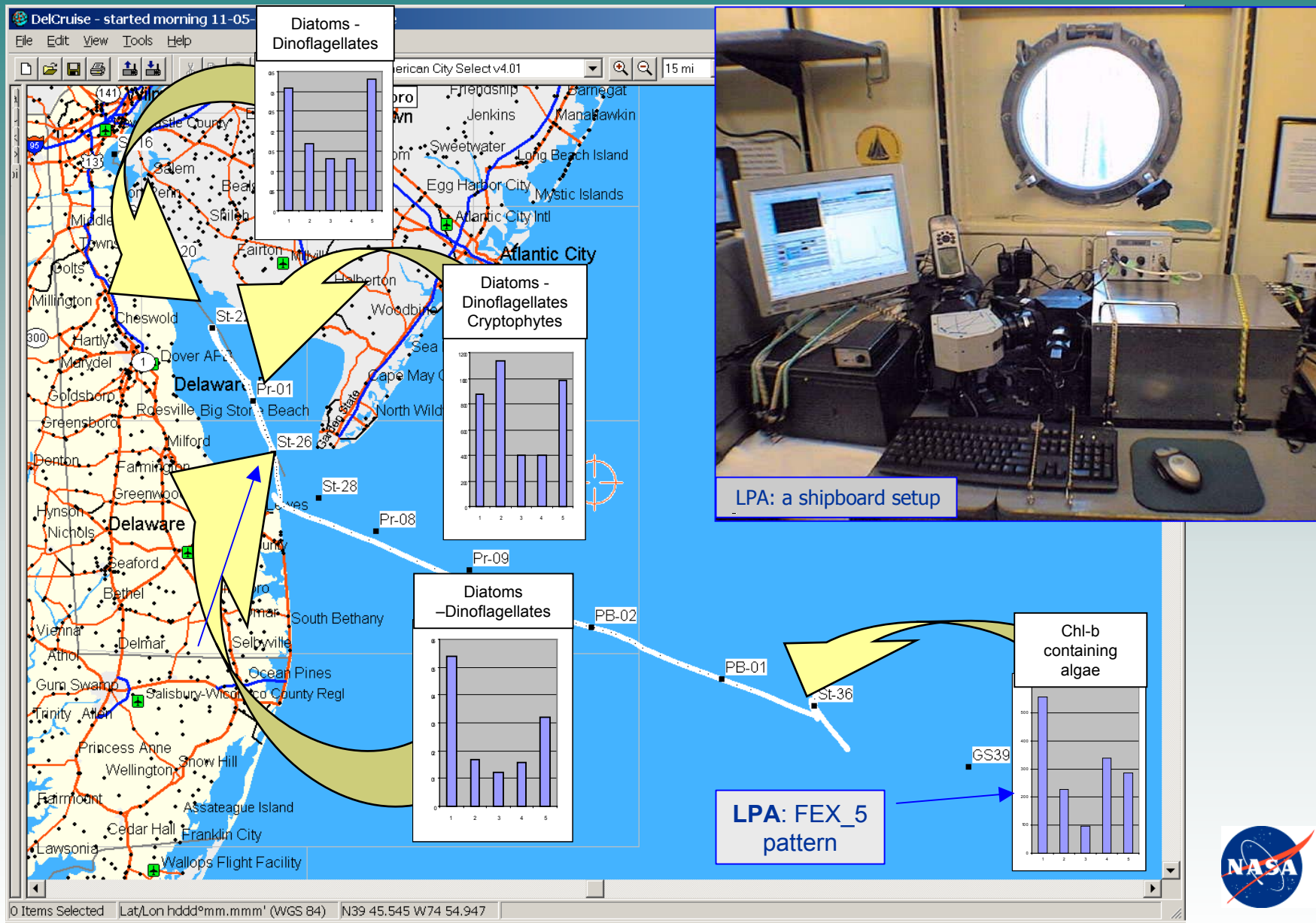
Prorocentrum minimum
Dinoflagellate

Storeatula major
Cryptophyte

Porphyridium cruentum
Rhodophyte

FEX_5 signatures of phytoplankton and cyanobacteria measured with Laser Phytoplankton Analyzer (LPA). Bar patterns represent efficiency of Chl-a fluorescence excitation with laser excitation at: 1 – 473 nm; 2 – 532 nm; 3 – 639 nm; 4 – 651 nm; 5 – 666 nm

LPA Field Test: Variability in Taxonomic Composition



Advanced Fluorescence Characterization of Algal Species: Laser Excitation/Emission Matrix (LEEM) Analysis

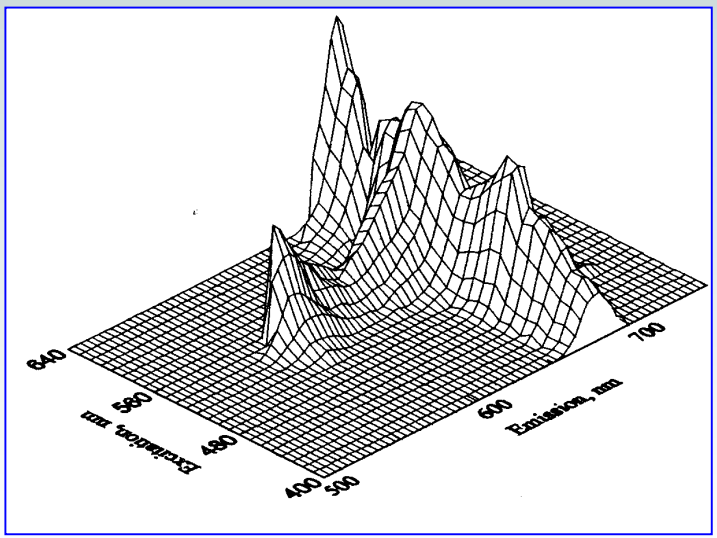
Optical Parametric Oscillator: Wavelength-tunable excitation (410-670 nm)

+

CCD Spectrograph: Hyperspectral Emission Analysis

LEEM pattern: 2D excitation-emission matrix analysis

=

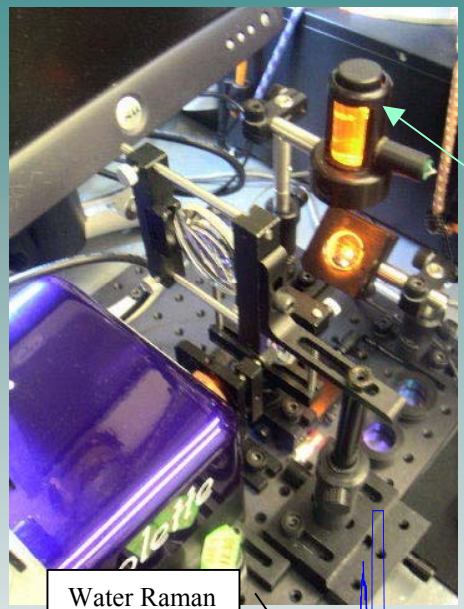


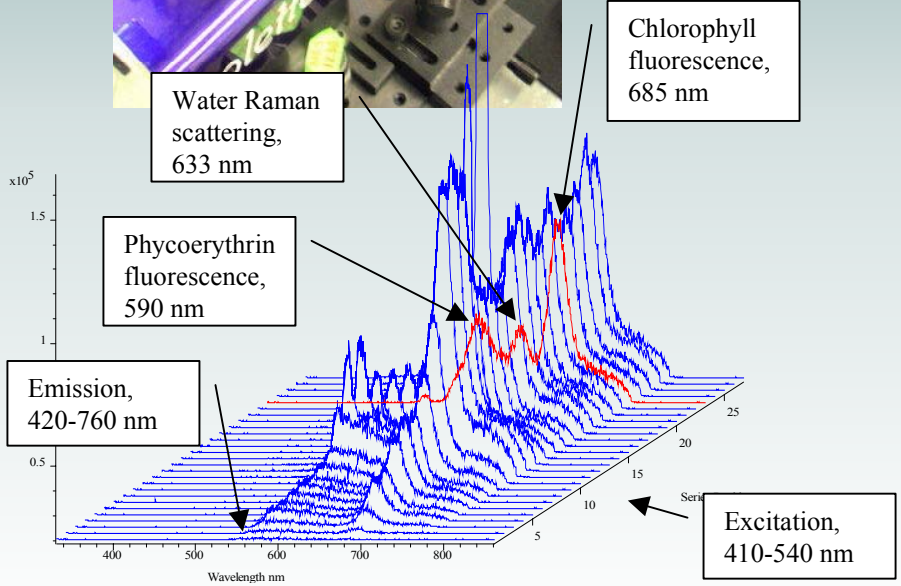
LEEM Analyzer:

- Fast excitation scans (410-670 nm)
- 'Instant' high-resolution FEM analysis
- High sensitivity to analyze natural phytoplankton populations
- Laboratory and shipboard operation

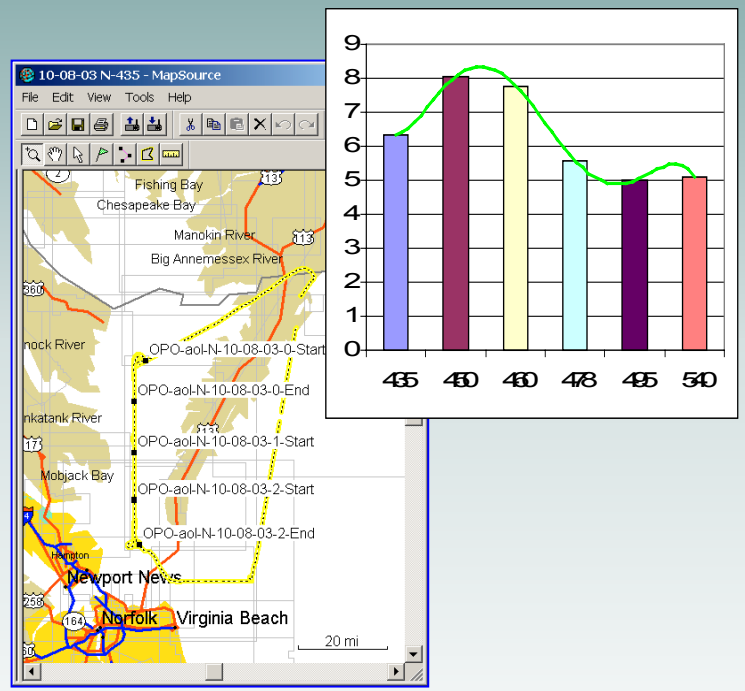
October 2003: Initial Shipboard LEEM Tests

A shipboard prototype system includes an **optical parametric oscillator** (OPO) for wavelength-tunable **excitation in 410-680 nm** range, and a **CCD spectrometer** for hyperspectral analysis of laser-stimulated pigment emission. The LEEM fluorometer was **tested in the Chesapeake and Delaware Bays** and Delaware River in October 2003.

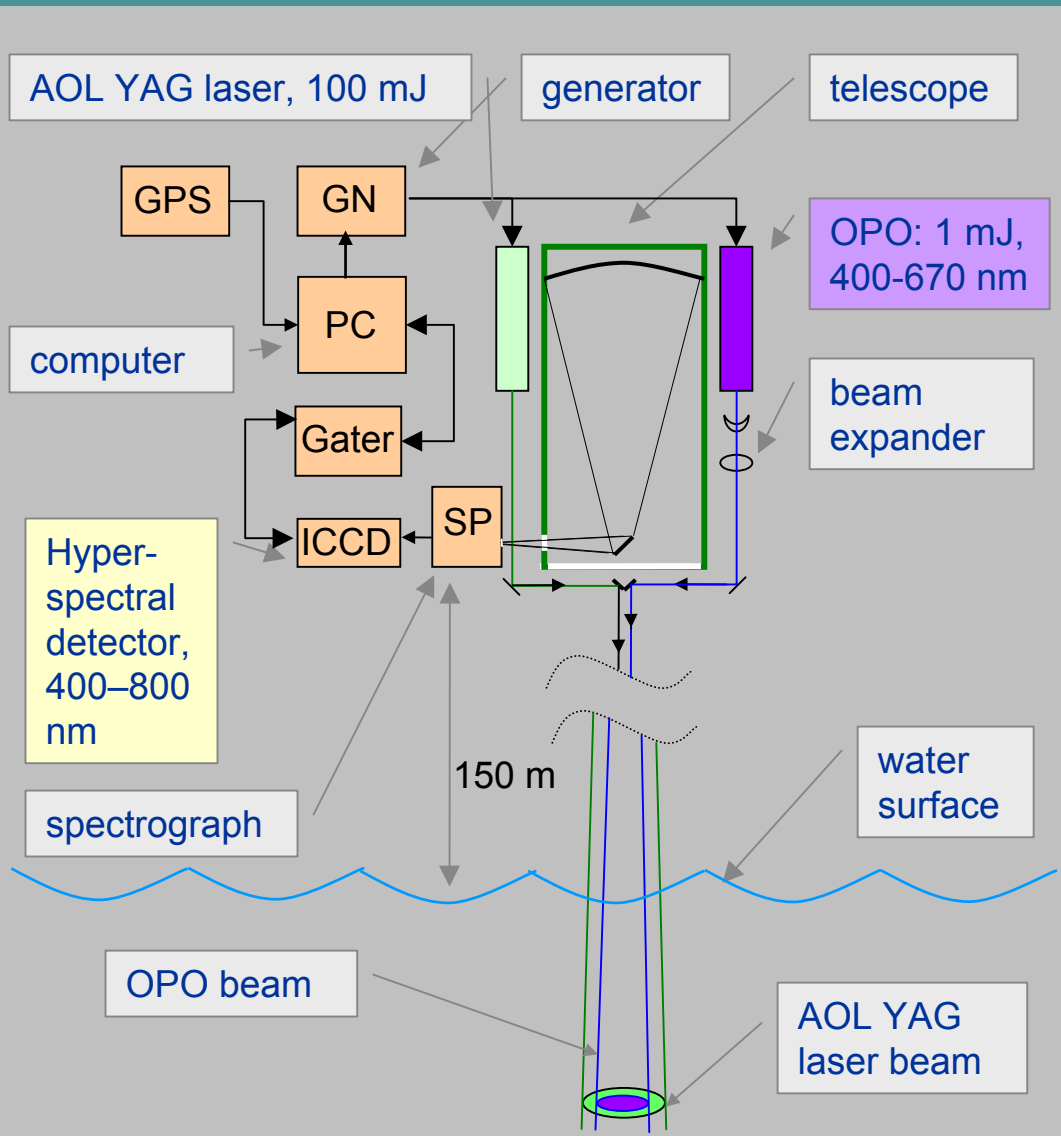




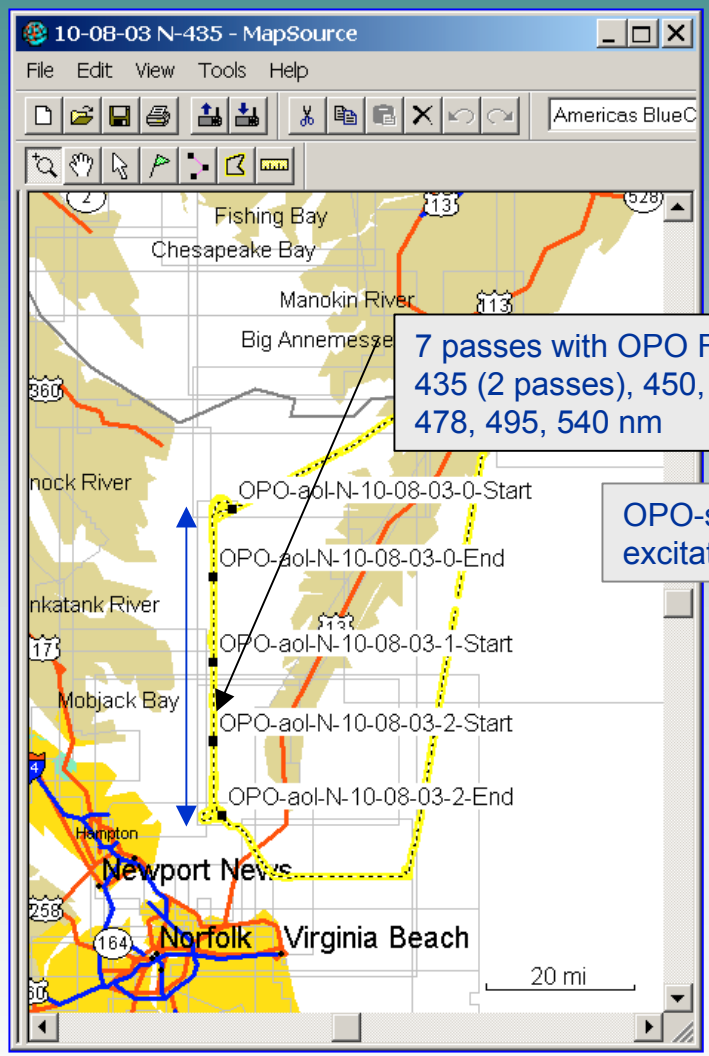
4. Prototype FEX/FEM LIDAR: Initial Airborne Tests



Prototype FEX/FEM LIDAR: Wavelength-Tunable Excitation & Hyperspectral Signal Detection

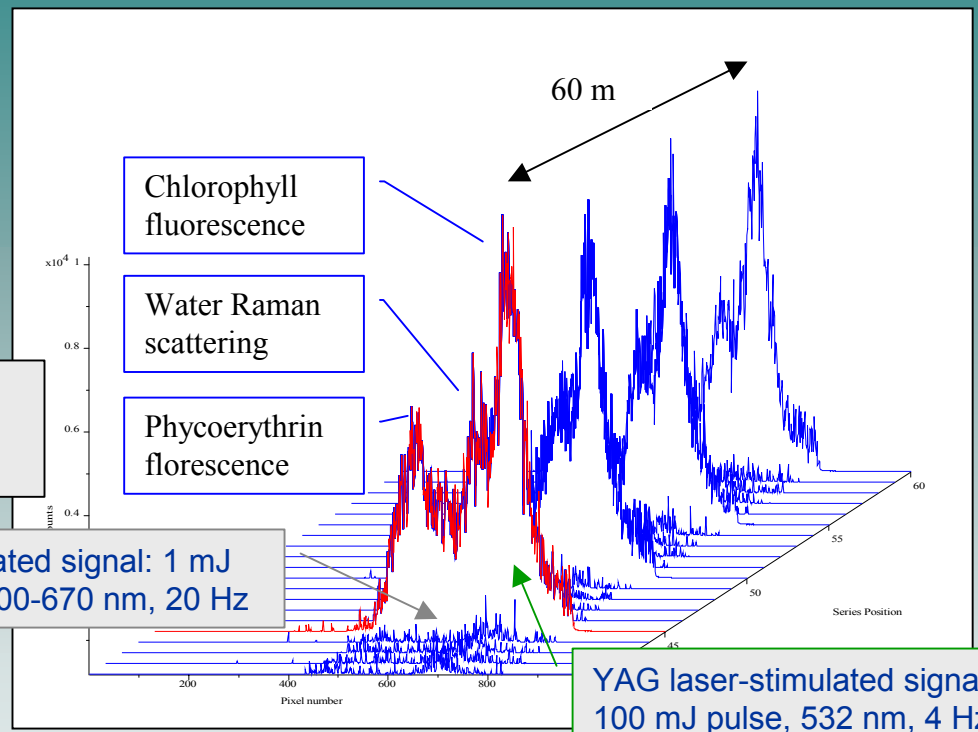


Prototype FEX/FEM LIDAR: Airborne Tests in the Chesapeake Bay and Middle Atlantic Bight, Oct. 2003



7 passes with OPO FEX = 435 (2 passes), 450, 460, 478, 495, 540 nm

OPO-stimulated signal: 1 mJ excitation, 400-670 nm, 20 Hz



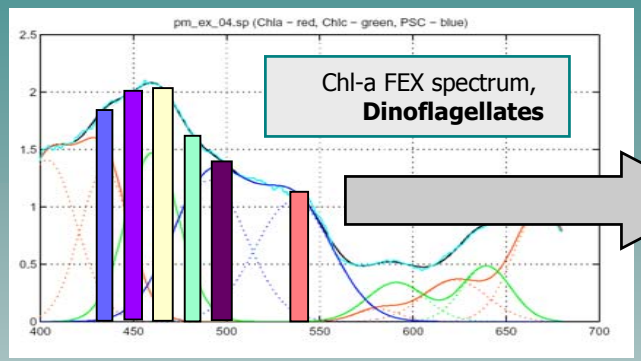
YAG laser-stimulated signal: 100 mJ pulse, 532 nm, 4 Hz

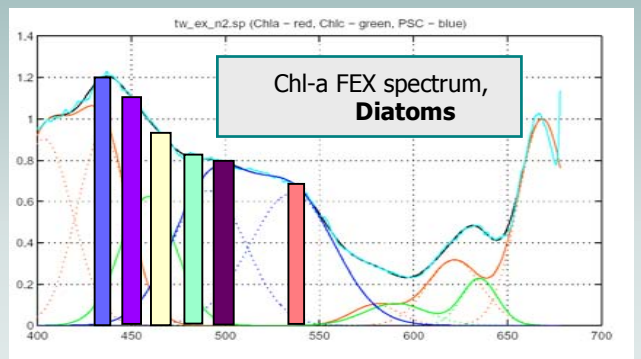
OPO/ICCD LIDAR measurement protocol:

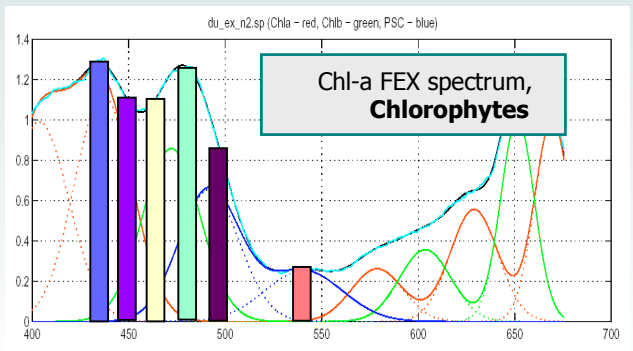
- OPO excitation: 435 (2 passes), 450, 460, 478, 495, 540 nm, 20 Hz rep.rate, 1 mJ per pulse
- AOL laser: 5 Hz rep. rate, 100 mJ per pulse
- ICCD camera: gated with 100 ns pulse, hyperspectral signal detection in 400-900 nm range

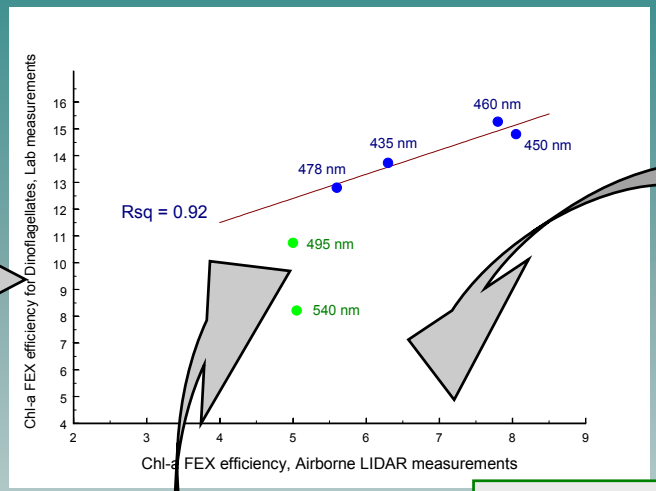
Prototype FEX/FEM LIDAR: Potential for remote identification of dominant algal groups

Laboratory FEX measurements:

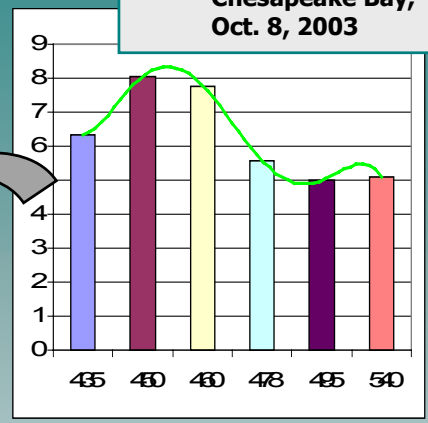


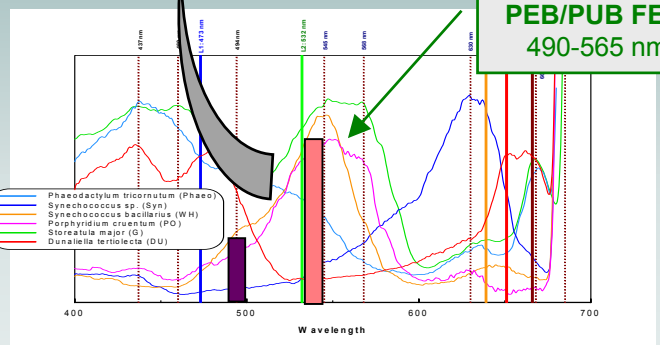




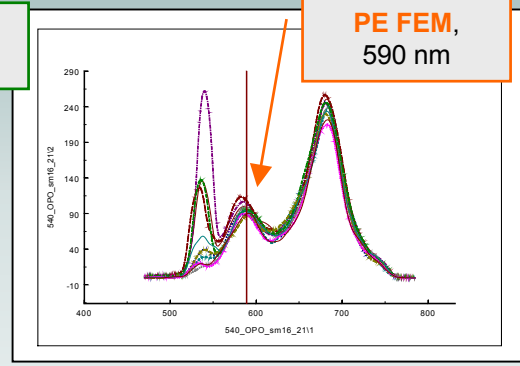


LIDAR FEX data, Chesapeake Bay, Oct. 8, 2003



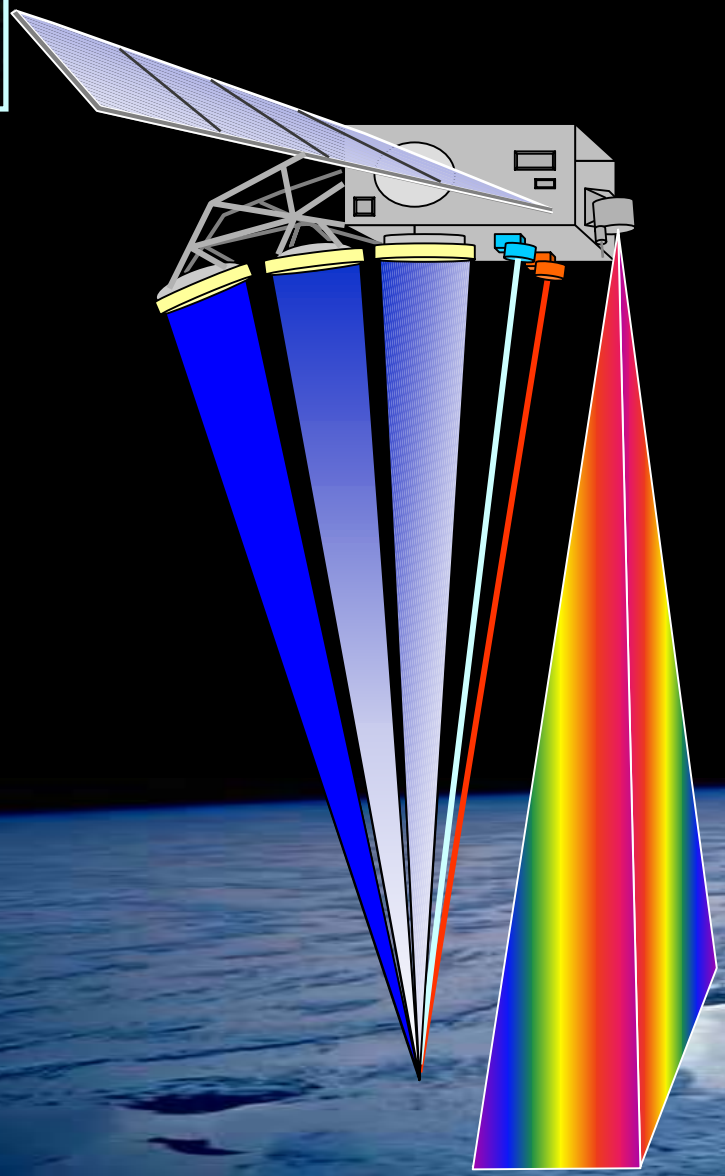


PEB/PUB FEX, 490-565 nm



PE FEM, 590 nm

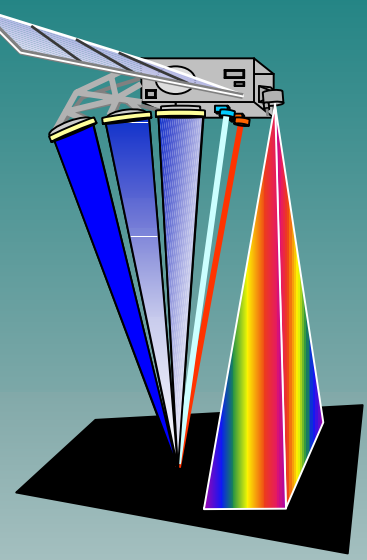
- **Blue FEX** (435-478 nm): **Dinoflagellate dominance** (high Chl-c)
- **Green FEX** (495, 540 nm): **Presence of phycoerythrin-** (PE) containing species with high PEB/PUB ratio
- **Orange FEM** peak (590 nm): **Cryptophyte dominance** among PE-containing groups (incl. cyanobacteria and rhodophytes)



PhyLM

5. The Physiology Lidar-Multispectral Mission: Feasibility of Active Fluorescence Measurements

Lidar Objectives

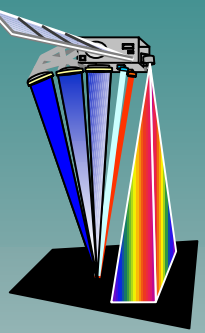


- Objective 1: Atmospheric corrections to enhance accuracy and precision of passive data
- Objective 2: Active measurements of backscattering, again supporting passive data
- **Objective 3: Active,** dark-side **fluorescence** to improve coastal & high-productivity data products
- Objective 4: Active measurements of beam attenuation, for improved phytoplankton carbon products

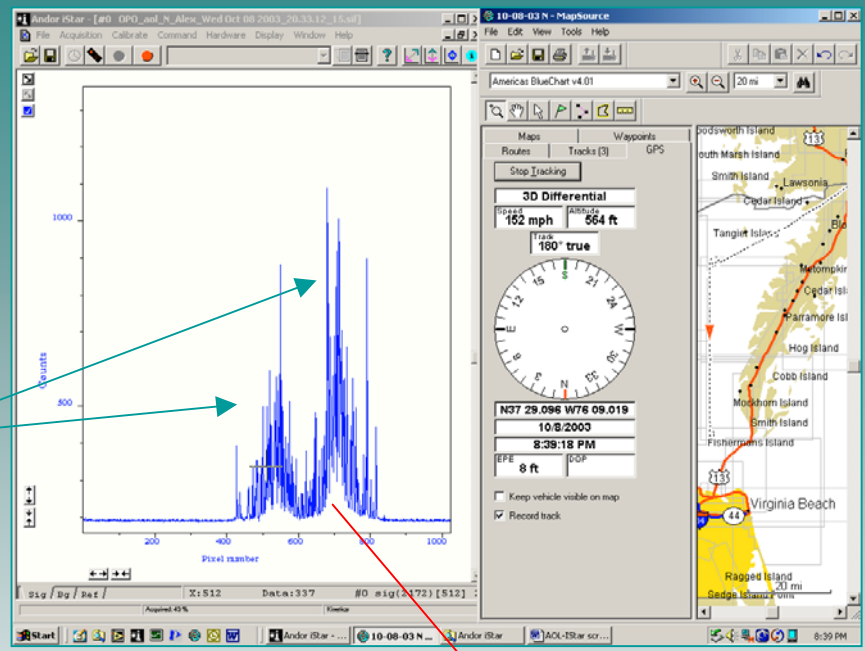
The PhyLM Mission

PhyLM vs. AOL: Lidar Fluorescence Measurements

The PhyLM Mission



AOL single-shot spectra of Phycoerythrin (590 nm) and Chlorophyll-a (685 nm) fluorescence; OPO excitation: 495 nm



	PhyLM	AOL	**PhyLM/AOL**
Laser:	1 J	1 mJ (OPO)	**10^3**
Telescope	5-50 m^2	0.05 m^2	**10^2-10^3**
Altitude	500 km	0.150 km	**$3.3 \cdot 10^3$**
$(Altitude)^{-2}$	~ $(1/500)^2$	~ $(1/0.15)^2$	**10^{-7}**

PhyLM Fluorescence Signal: Photons…

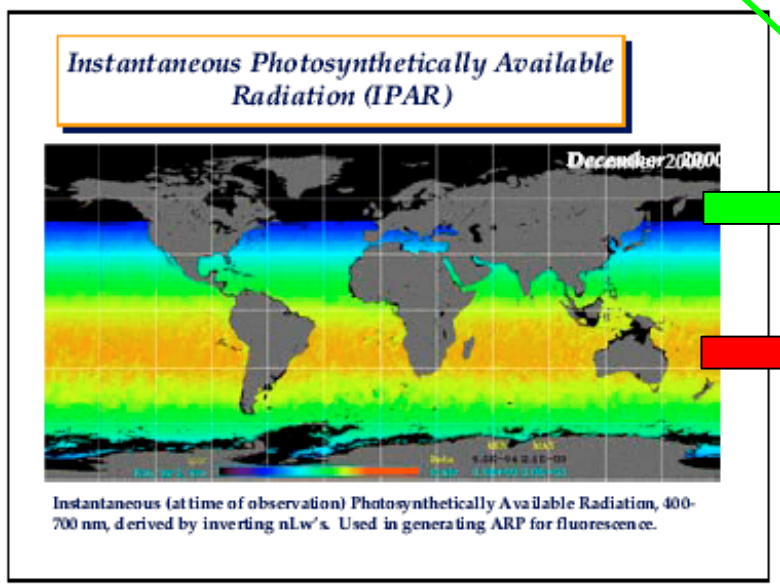
6. Assessment of Phytoplankton Physiology and Productivity from Natural Fluorescence : Bad News, Good News…

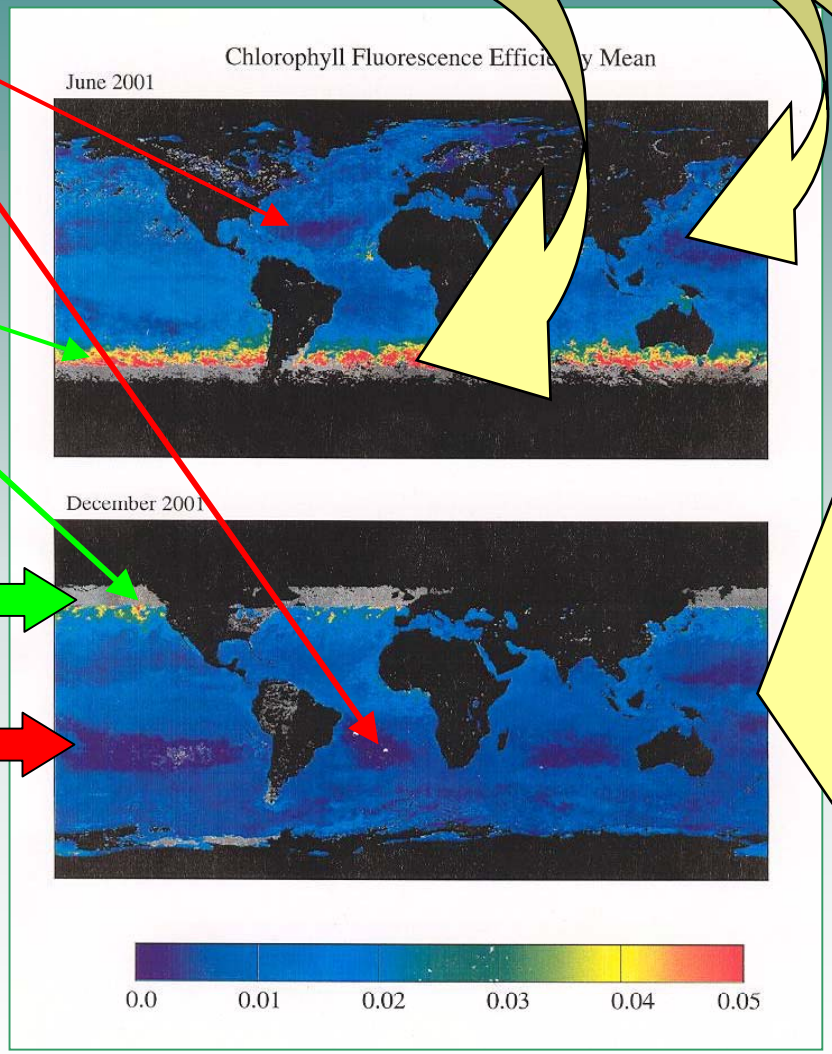
MODIS Natural Fluorescence : Good News (feasible in high latitudes), Bad News (no global assessments of phytoplankton physiology)

$$FLH / ARP = \text{`CFE`} = K_f/(K_\Sigma + C_A(PAR)\cdot f\cdot K_P + K_{NPQ}(PAR))$$

High PAR:
CFE is controlled by **NPQ**;
Physiology is non-retrievable
(don't shoot the messenger)

Low PAR:
`CFE` depends on physiology, f, and PAR





Fluorescence Assessment of Primary Productivity (PP): Accounting for Variability in Photosynthetic Quantum Yield

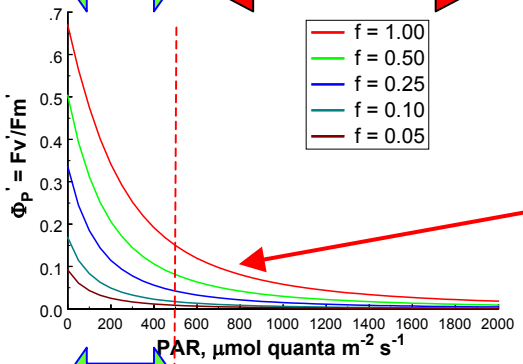
Fluorescence assessment of PP: $PP = ARP/Z_{685} \cdot \Phi_P'(PAR, f) \cdot \phi_e \cdot M_c$

PSII photochemical efficiency under ambient light:

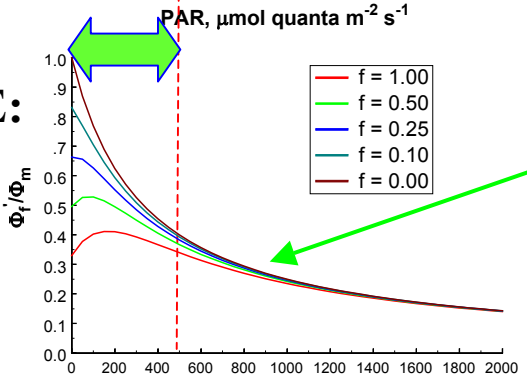
$\Phi_P'(PAR, f) = F_v'/F_m' =$

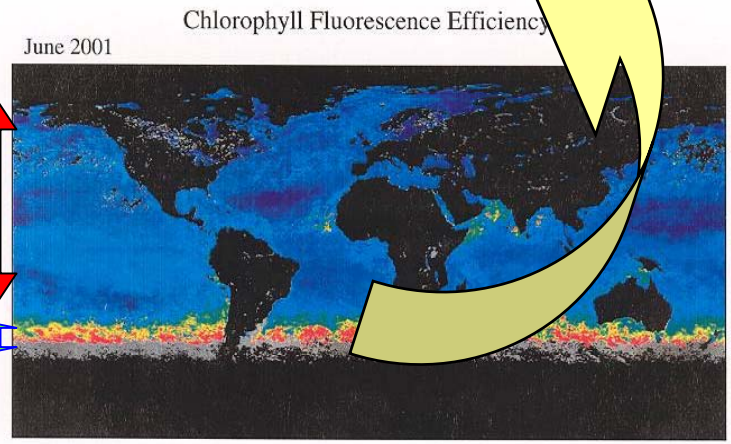
$C_A(PAR) \cdot f \cdot K_P / (K_\Sigma + C_A(PAR) \cdot f \cdot K_P + K_{NP}(PAR))$

Φ_P' :



CFE:





High PAR: $\Phi_P'(PAR, f)$ drops, but still **depends on physiology**, which is **non-assessable** because the **CFE dependence on physiology is weak**; still **may work regionally** with airborne LIDAR input on '**f**'

Low PAR (high latitudes): should work!

View from Moon: Global Assessment of Algal Physiology & Productivity from Natural Fluorescence

Moon-based Earth-observing system:

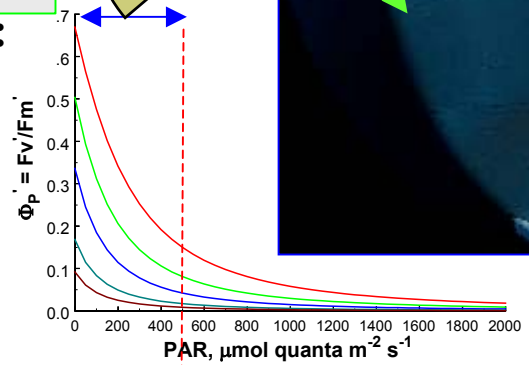
• Continuous dynamic spectral **imaging of natural fluorescence under low-light regime** can provide **global assessment of phytoplankton physiology, f,** for improved estimates of primary productivity.

• A biophysical model, which accounts for algal physiological variability, can be utilized:

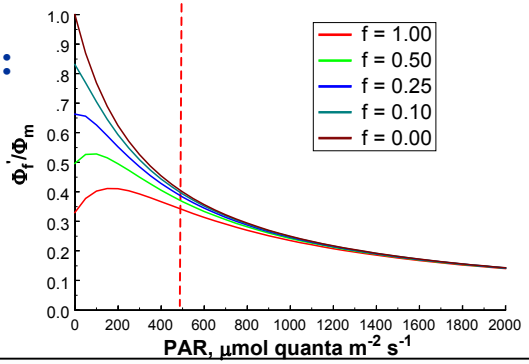
$$PP = ARP/Z_{685} \cdot \Phi_P'(PAR, f) \cdot \phi_e \cdot M_c$$

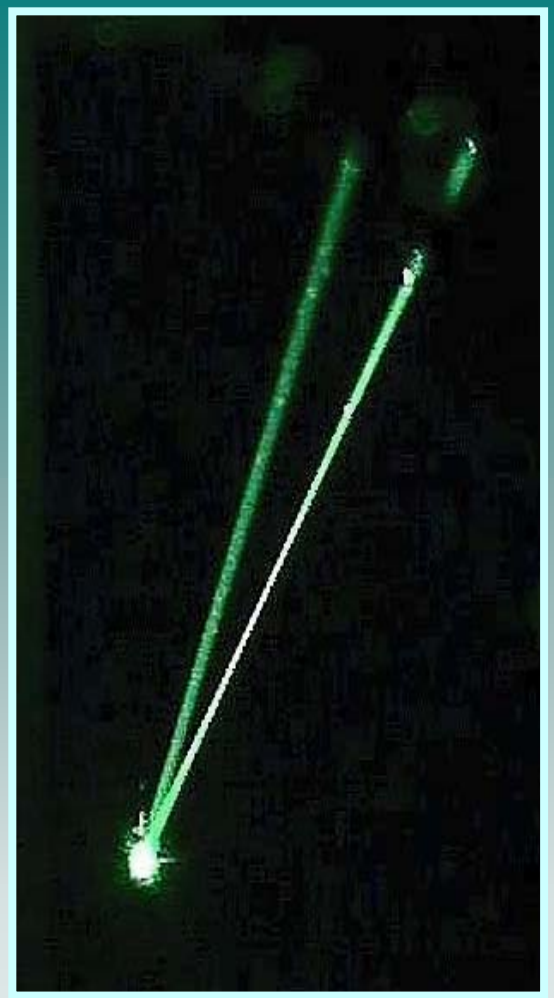
Alternative platforms: **Geostationary satellites**

Φ_P' :



CFE:





Concluding Note:

Spring 2004:
No AOL field measurements…

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R.N. Swift[2] , J.K. Yungel[2], P.E. Lyon[2]

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Wallops Flight Facility, chekaluk@osb.wff.nasa.gov