In situ observation capabilities and strategies for phytoplankton composition

to support development and validation of satellite PFT algorithms

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IOCS 2015 – Breakout “Remote Sensing of Phytoplankton Composition – Possibilities, Applications and Future Needs”
San Francisco, CA 16 June 2015

Overview

IOCCG PFT Report No. 15
Chapter 2: In situ methods of measuring phytoplankton functional types.
Sosik, Sathyendranath, Uitz, Bouman & Nair.

Assessing State-of-the-Art

- Strengths / Limitations
  - HPLC pigments
  - Microscopy
  - Flow Cytometry
  - Genetic Analysis
- Cross-cutting challenges
  - Space/time mis-match with satellite observations
  - Abundance vs. biomass
  - Cell size characterization & biovolume estimation
  - Biomass metrics
    - biovolume vs. carbon vs. pigment / chlorophyll

HPLC - High Performance Liquid Chromatography

"Chemotaxonomy" (e.g., Mackey et al. 1996)

Ratios of accessory pigments

➔ infer relative contribution of major groups to total chlorophyll

Diatoms
Dinoflagellates
Prymnesiophytes
Pelagophytes
Cryptophytes
Chlorophytes
Prochlorophytes
Cyanobacteria

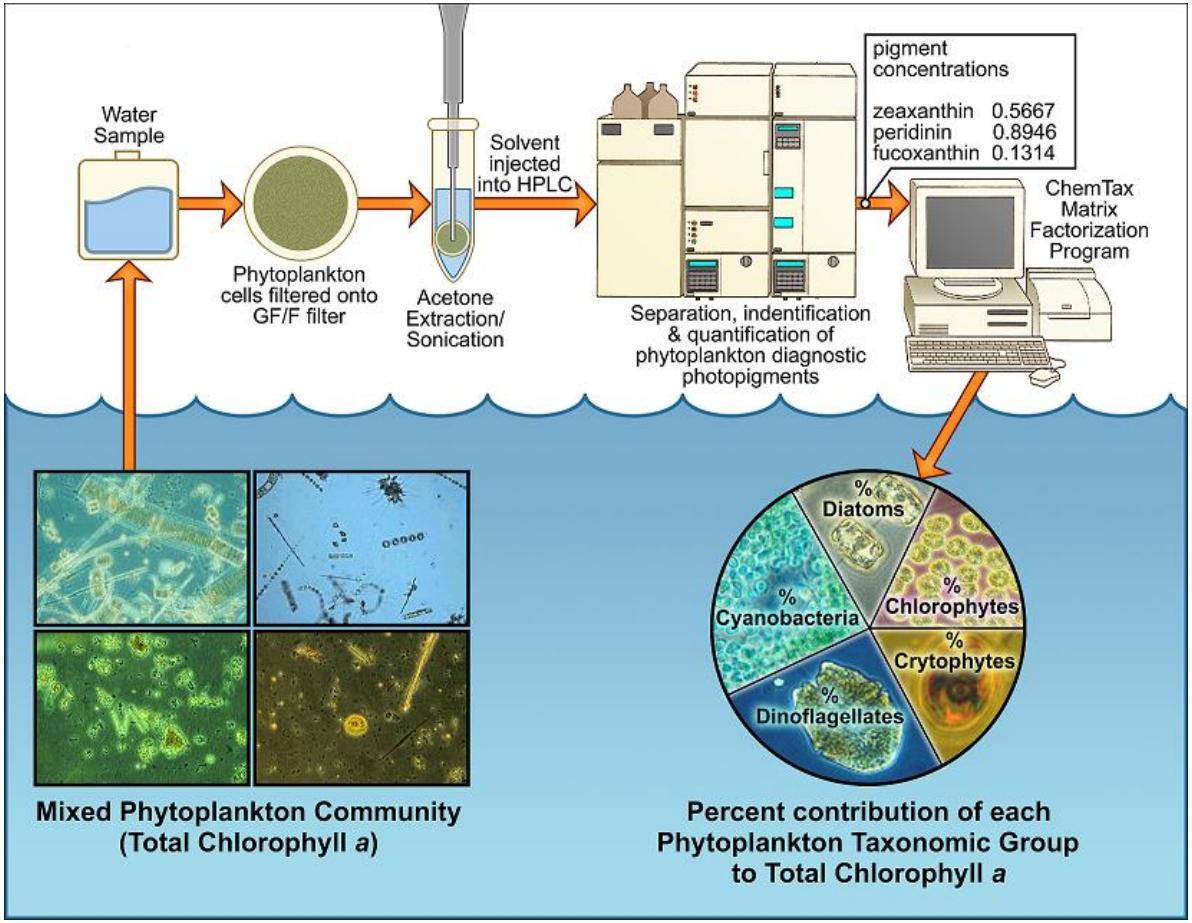


Figure credit: Joyner and Paerl (UNC)

HPLC

Strengths

- Highly precise
- Detection limits adaptable
- Routine sample collection and preservation
- Biomass assessment as pigment
- Taxonomic expertise not required

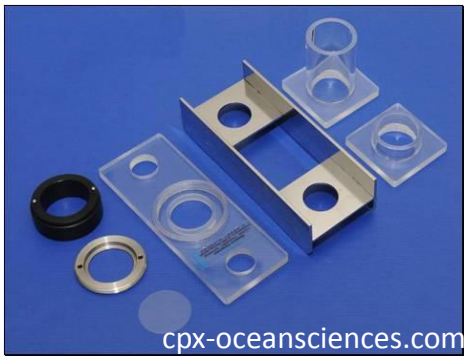
Limitations

- Specialized equipment and technical needs
- Ambiguities in marker pigments
- Sensitive to assumptions about pigment ratios
  (inter- and intra-group variability)
- Taxonomic detail modest (group level)
- Cell size only indirectly inferred

Microscopy

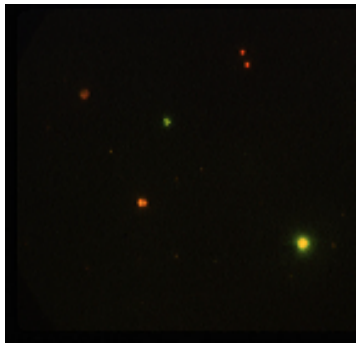
Light microscopy
"Utermöhl method"
preserved samples
settled in chamber
cell counts across
known size fields
- **Effective for microplankton**

inverted microscope



settling chambers

Epifluorescence microscopy
filtered samples
exploits inherent fluorescence
chlorophylls
phycobiliproteins
- **Effective for picoplankton**



<u>Continuous Plankton Recorder</u>
phytoplankton by light microscopy
directly on preserved sample silk

<u>Electron microscopy</u>
provides adequate detail for some
difficult taxa
trade-off increased cost & time,
reduced throughput

Microscopy

Strengths

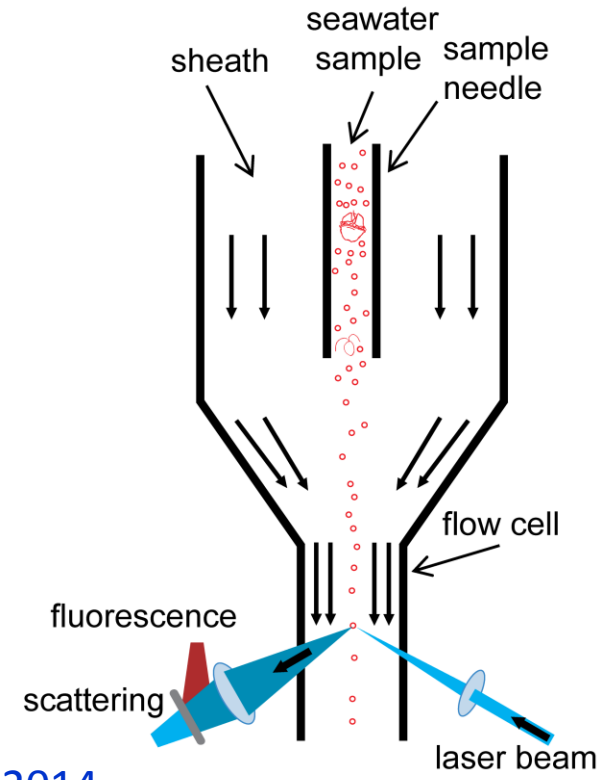
- Taxonomic detail high
- Detection limits adaptable
- Cell size and taxon accessible

Limitations

- Taxonomic expertise required
- Time consuming
- Subject to preservation artifacts / biases
- Multiple methods for full size range
- Many small cells difficult to identify

Flow Cytometry

Conventional



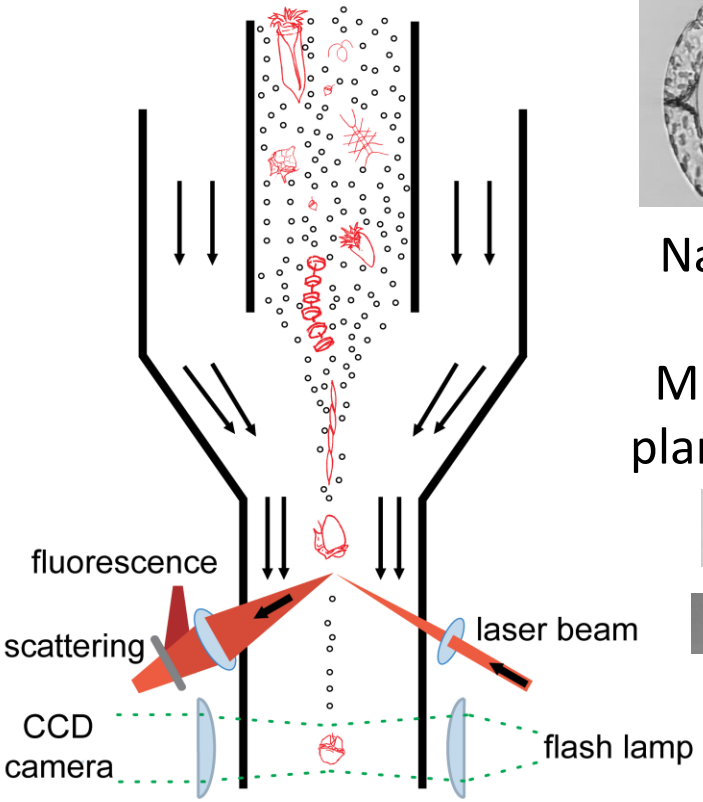
Pico-
&
Nano-
plankton

Sosik et al. 2014

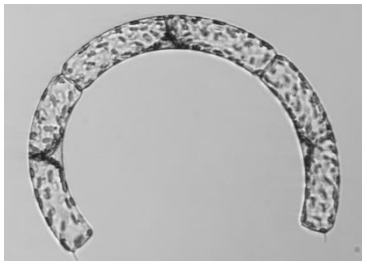
Single cell, typical measurements :

- Chlorophyll fluorescence
- Light scattering (forward, side angle)
- Phycoerythrin fluorescence

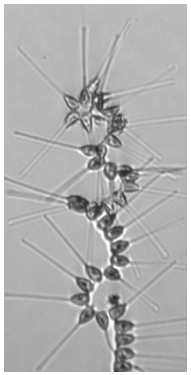
Imaging-in-Flow



Same as conventional
Plus images
~ 1 μm resolution



Nano-
&
Micro-
plankton



+ sorting flow cytometry for specialized analysis

Flow Cytometry

Strengths

- Analysis automatic and rapid
- Precise and quantitative
- Some taxonomic detail for selected groups
- Optical cell size estimation possible
- In situ instruments available

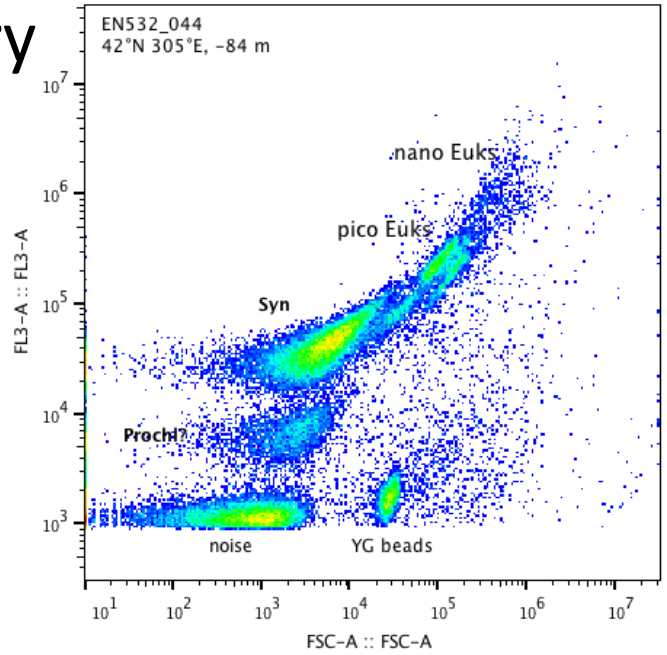
Limitations

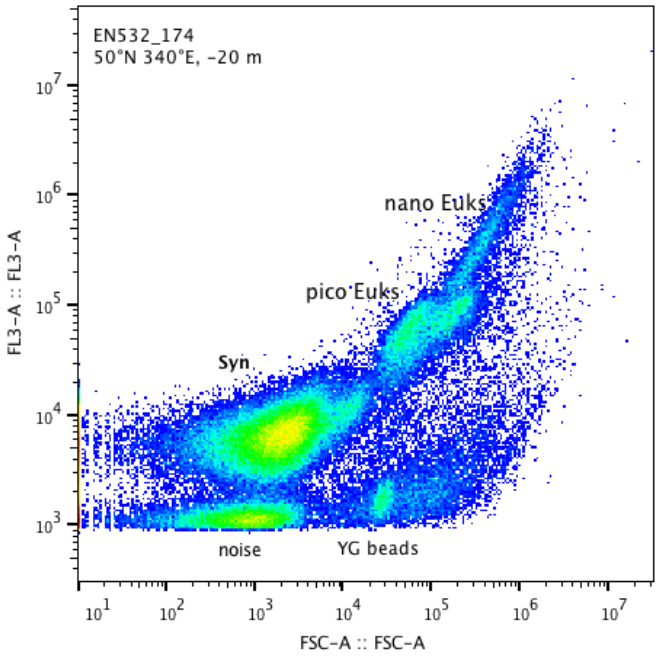
- Many taxa not separable
- Relatively expensive / delicate instruments
- Requires some user expertise
- Specialized methods / instruments for full size range

Flow Cytometry

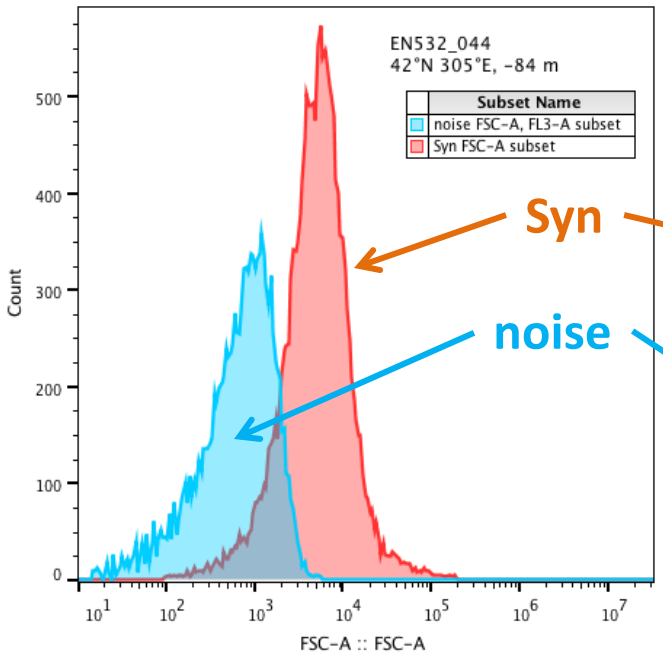
BD Accuri C6 flow cytometer

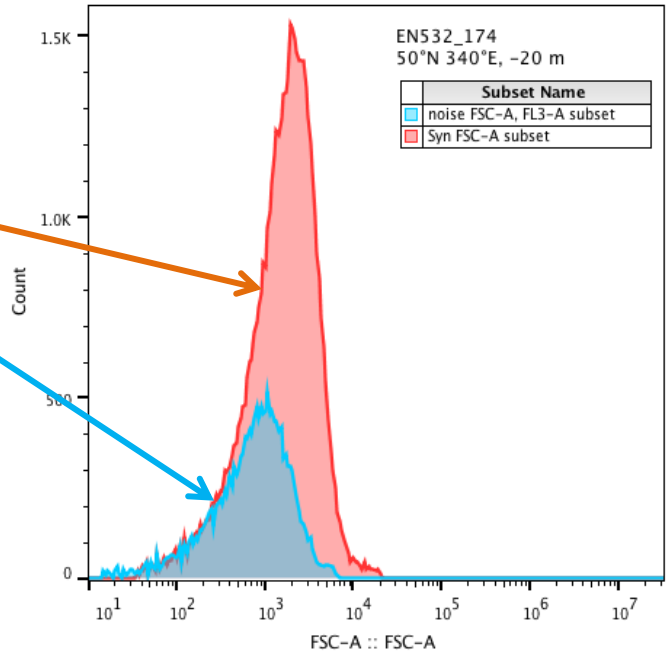
Challenges:
sensitivity issues for
- *Prochlorococcus* detection
- *Synechococcus* sizing





Van Oostende & Ward unpub





Genetic Analysis

Sequence targets
Typical diversity markers
16S/18S rRNA,
hypervariable regions, etc.
Other functional genes
C fixation, N assimilation, etc.

Wide range of methods, selection of interest:

Clone Libraries

PCR-based assay
presence of sequence types, whether known or unknown

Microarrays

hybridization to selected specific sequences
presence/absence/relative abundance for every sequence type on array
faster, higher throughput

High throughput sequencing, ribosomal marker surveys

PCR-based assay, no cloning (lower bias than libraries)
presence / relative abundance, whether known or unknown
sequence everything, search for info of interest

Metagenomics, transcriptomics, proteomics

presence / relative abundance
sequence everything, search for info of interest
potential for functional information
many challenges for eukaryotes

Genetic Analysis

Strengths

- Taxa can be targeted with high degree of specificity
- Particular functions can be targeted directly
- High throughput methods exist
- Specialized taxonomic expertise not required
- In situ tools emerging

Limitations

- Probe / primer availability can be limited
- No direct cell size information
- Method development and testing time consuming
- Methodological biases can be difficult to characterize
- Complex interpretation
- Dependence on sequence database content

Genetic Analysis

Clone libraries
- English Channel
- Monterey Bay

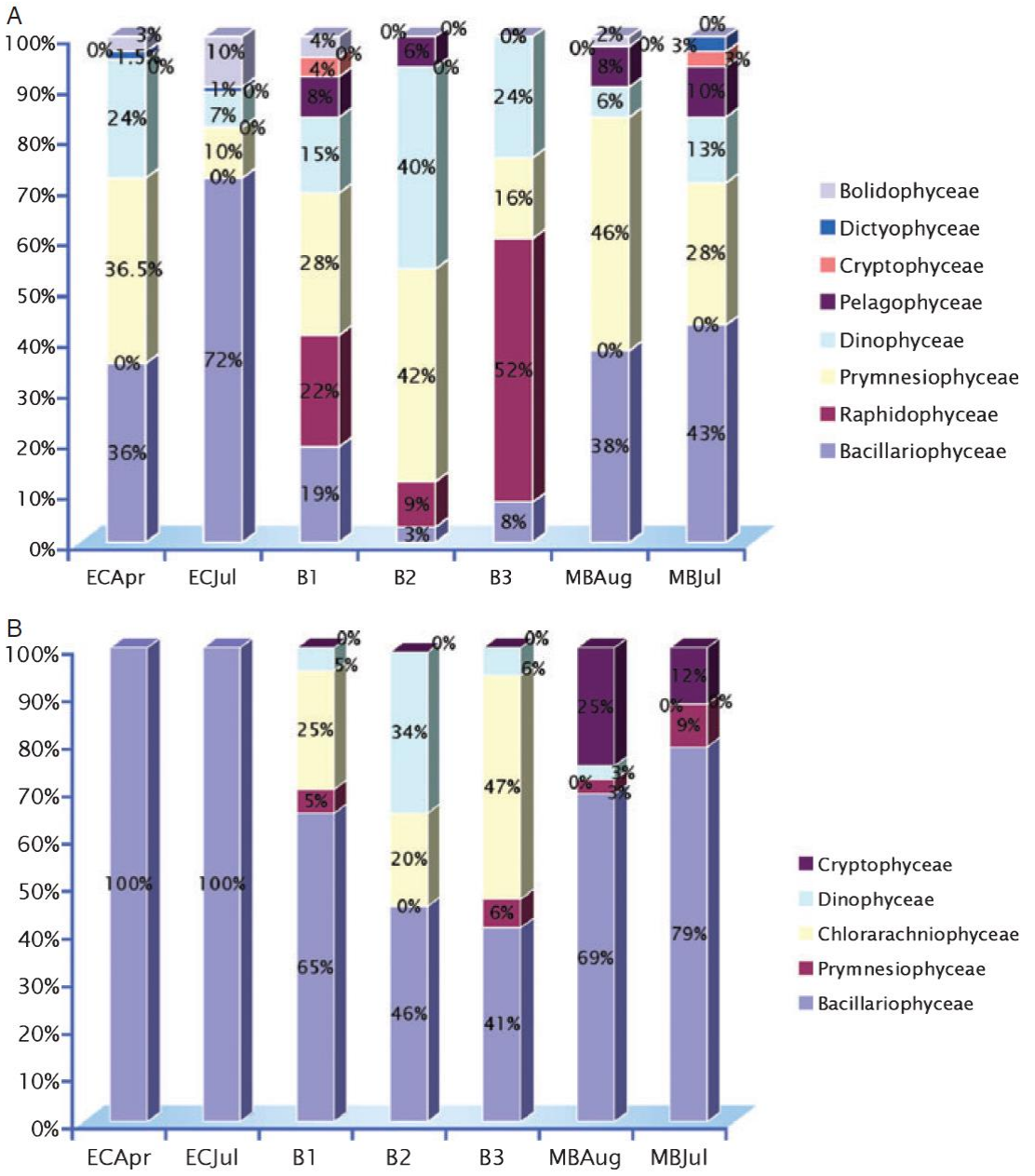
Bhadury & Ward 2009

rbcL
LSU of RUBISCO gene

NR
nitrate reductase gene

(NR primer – diatom bias)

→Composition estimates depend on gene target



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Biomass estimation

- Microscopy

  Cell dimensions → cell volume → cell C

  Relies on standard shape assumptions; literature-based C:volume relationships, time-consuming manual sizing of relatively few cells

- Flow cytometry, automated cell imaging

  Cell dimensions → cell volume → cell C

  similar approach, automated analysis provides quantitative information for many cells

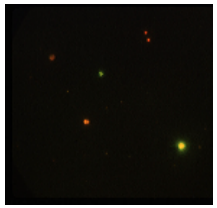
Biomass estimation

$$\text{Carbon} = \sum_i C_i$$

$$C_i = f(V_i)$$

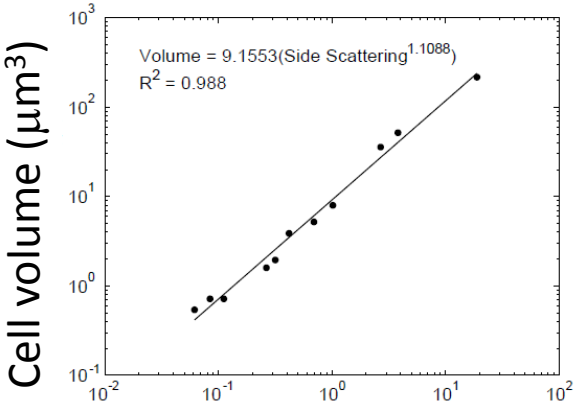
e.g., Menden-Deuer and Lessard 2000

Pico/nanoplankton



FlowCytobot

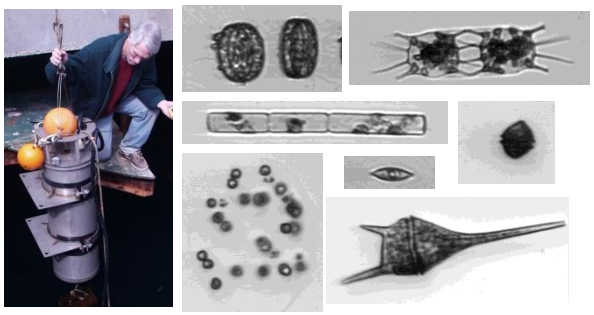
Volume from laser scattering



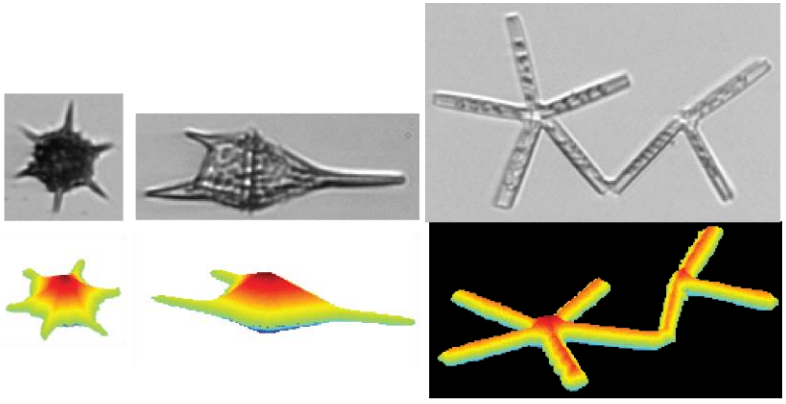
Light scattering

Olson et al. 2003

Nano/microplankton



Imaging FlowCytobot
Olson and Sosik 2007

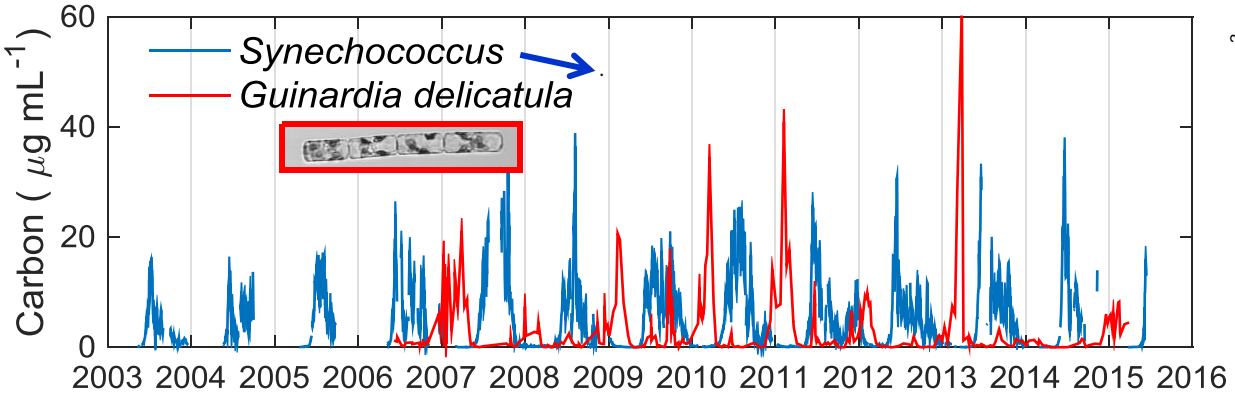


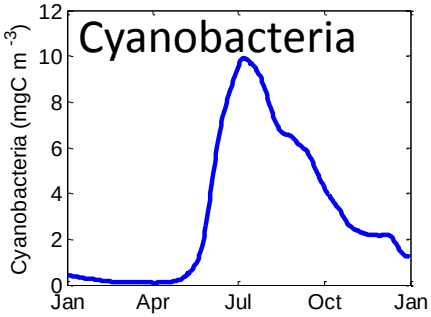
Volume from image analysis
"distance map" approach

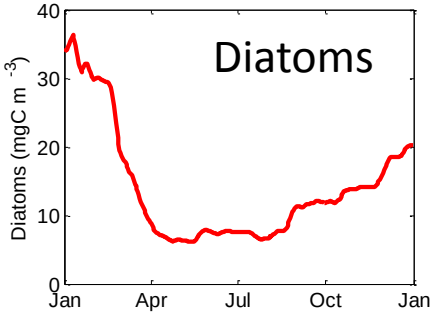
Sosik and Olson 2007
Moberg & Sosik 2012

Biomass estimation

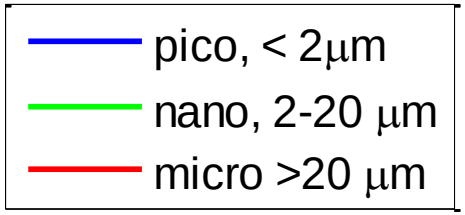
Individual cells → Taxa → Communities

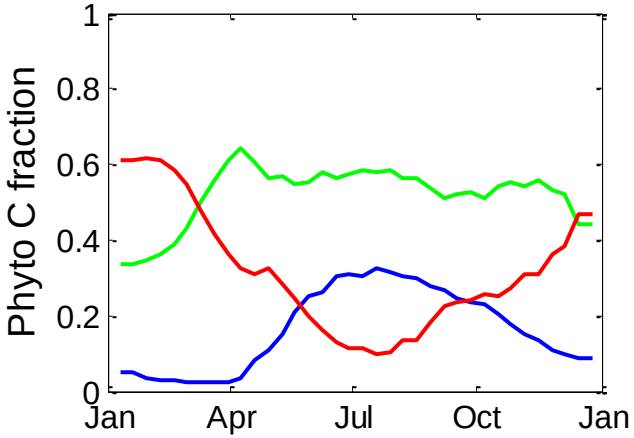






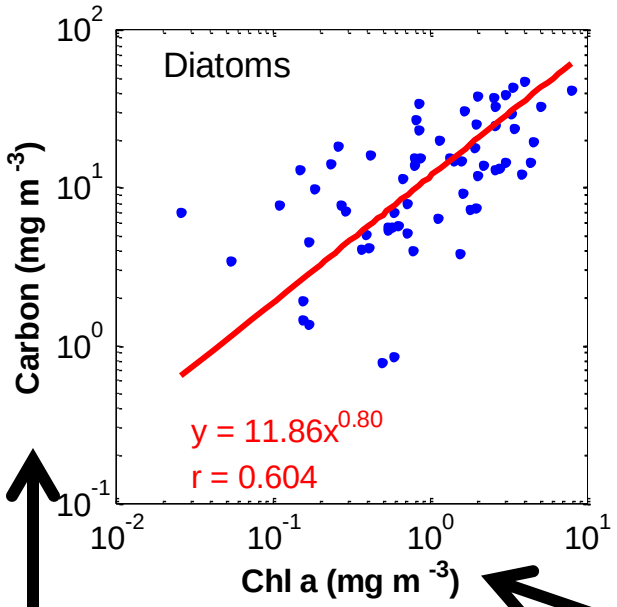
Individual cells → Size-classes → Communities



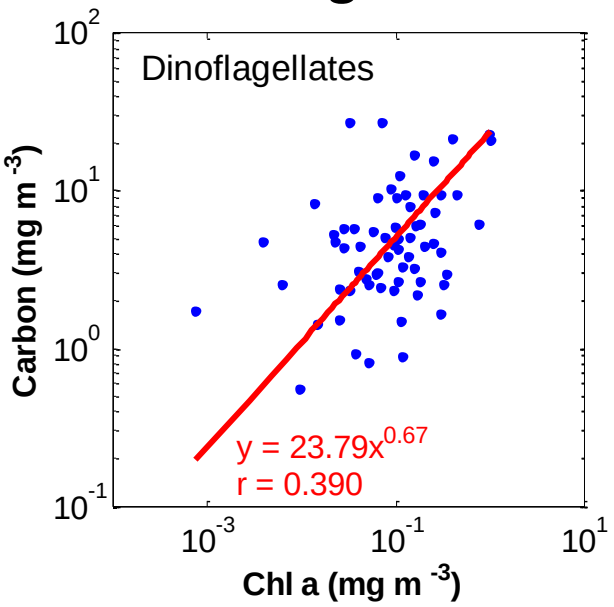


Biomass estimation – comparing metrics

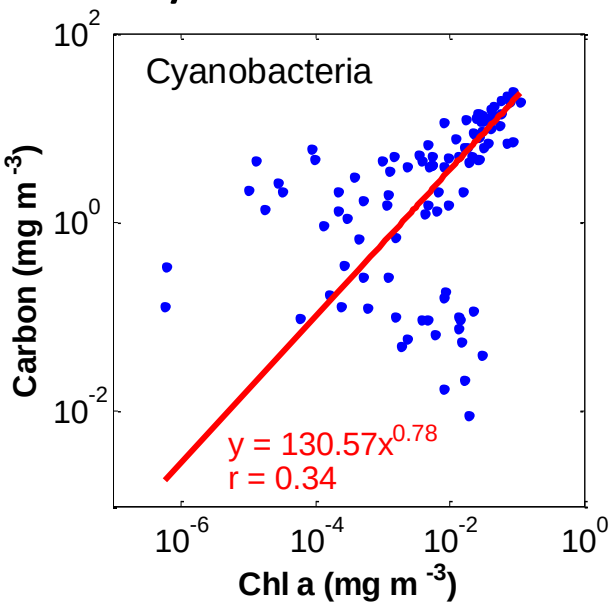
Diatoms



Dinoflagellates



Cyanobacteria



Merged FCM, imaging FCM

CHEMTAX from HPLC

Implied Carbon : Chl variations very large

diatoms ~10

dinoflagellates ~25

cyanobacteria ~250

Discussion of Future Directions

HPLC / Microscopy / Flow Cytometry / Genetic Analysis

Cross-cutting challenges

- **Space/time mis-match with satellite observations**
- **Abundance vs. biomass**
- **Cell size characterization & biovolume estimation**
- **Biomass metrics**
  - **biovolume vs. carbon vs. pigment / chlorophyll**

Which metrics will best serve which questions?

What methods are required for those metrics?

Recommendations for observations going forward?