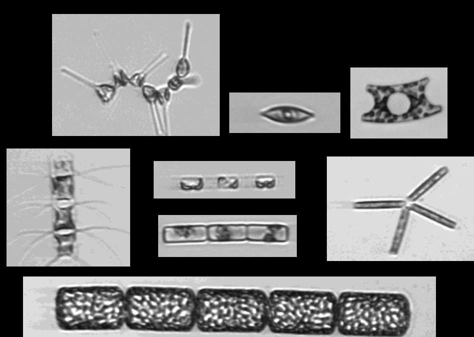
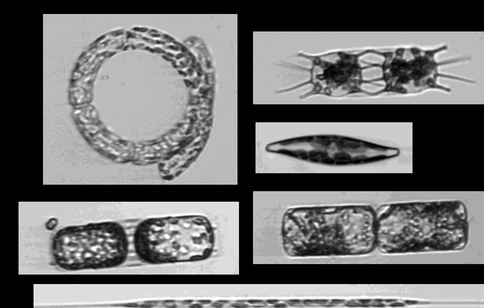
Resetting the baseline for phytoplankton in situ measurements:

Can we routinely measure phytoplankton diversity and size?

Heidi M. Sosik





International Ocean Colour Science Meeting,15-18 May 2017, Lisbon, Portugal

Current baseline for in situ phytoplankton measurements

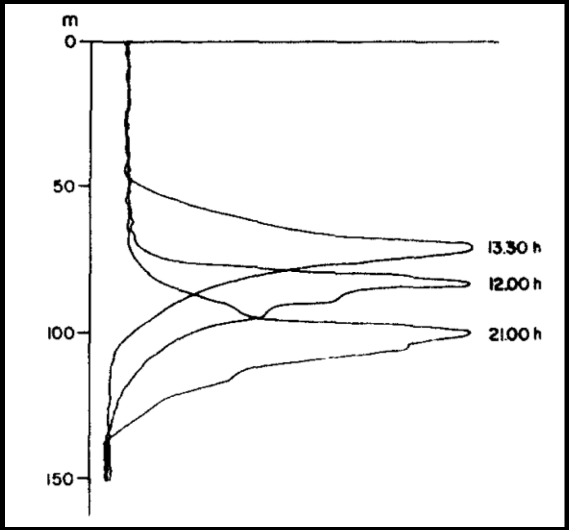
Advent of fluorometry in 1960s and 1970s

eBay

Lorenzen, 1966

Bedford Institute of Oceanography

Herman and Denman 1977

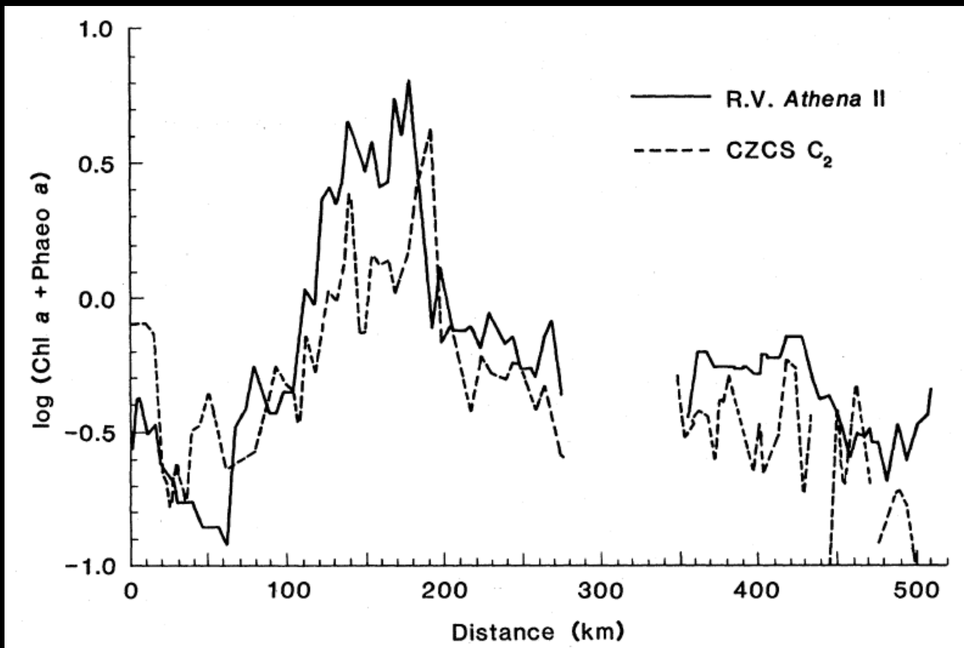


Gieskes et al. 1978

Current baseline for in situ phytoplankton measurements

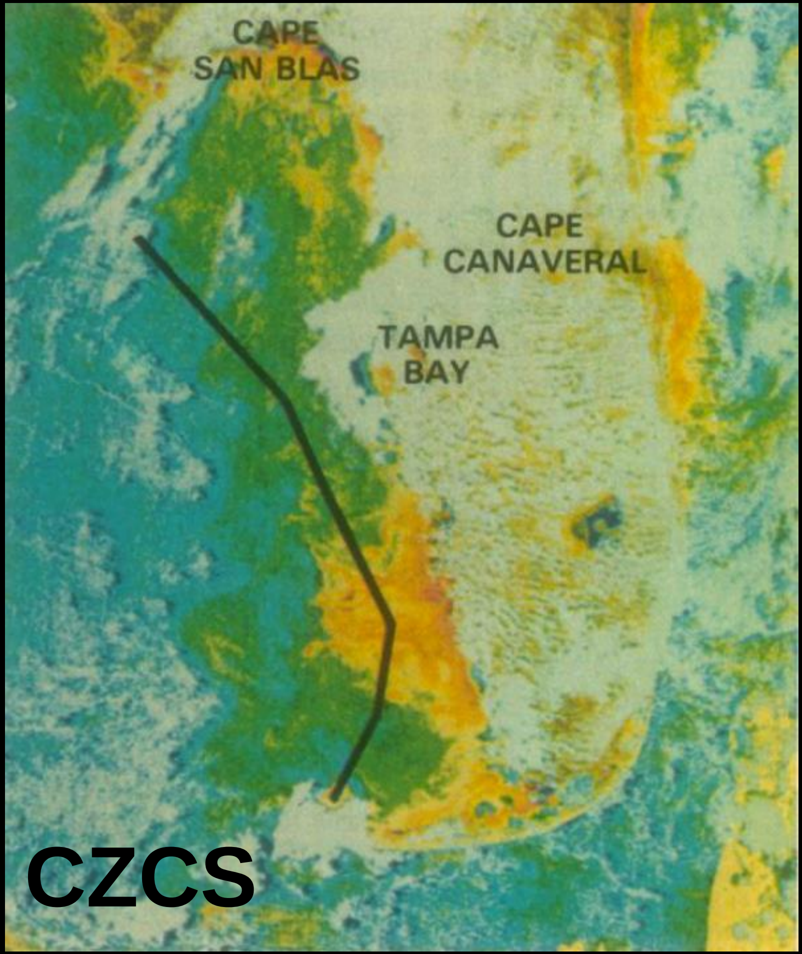
Chlorophyll vs. distance along track

Gordon et al. 1980



eBay

US$39.00 !



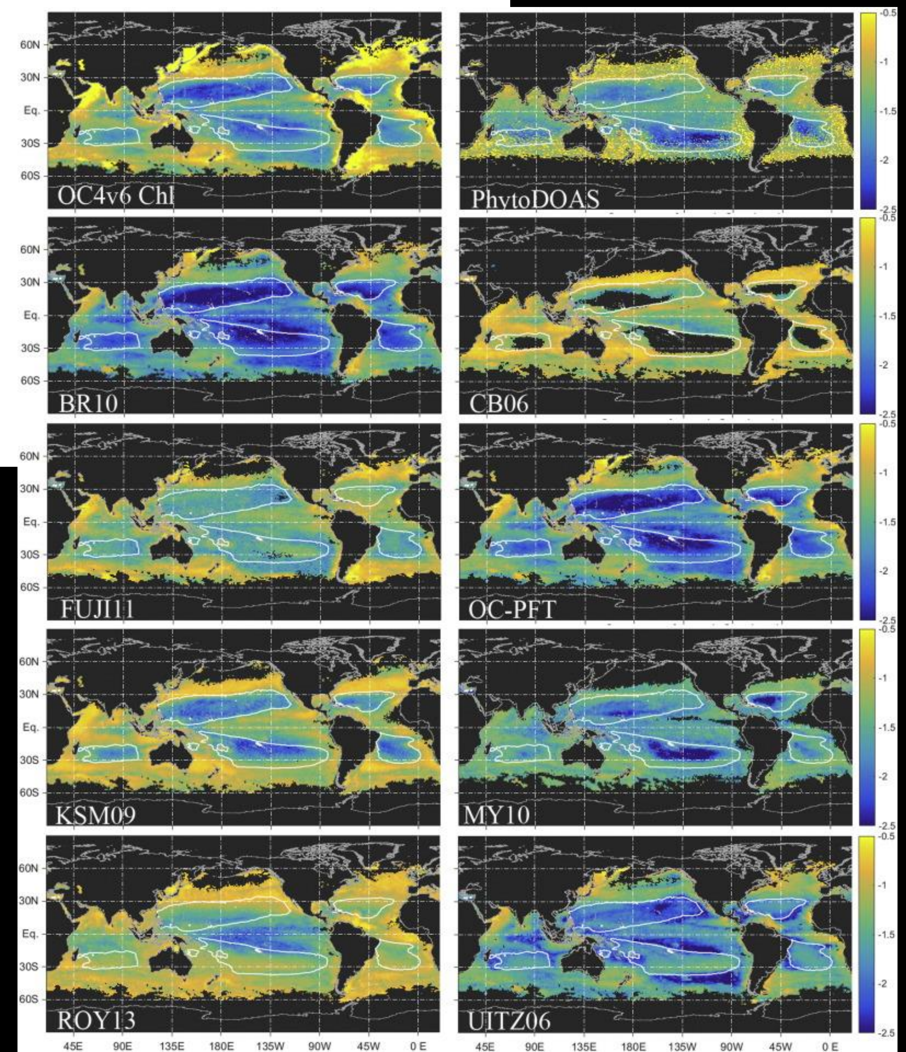
Beyond chlorophyll…

Reports and Monographs of the International Ocean-Colour Coordinating Group

An Affiliated Program of the Scientific Committee on Oceanic Research (SCOR)
An Associated Member of the (CEOS)

IOCCG Report Number 15, 2014

Phytoplankton Functional Types from Space



9 algorithm comparison

Wide discrepancy in seasonality for “large” / diatom-like PFT

Kostadinov et al. 2017

Beyond chlorophyll….

Can we routinely measure

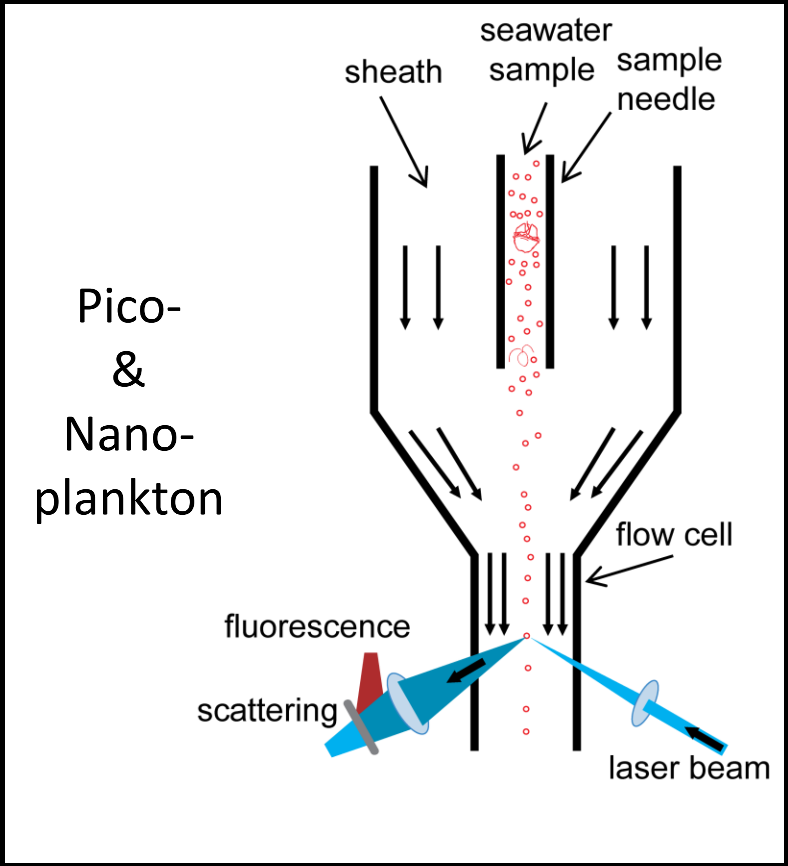
phytoplankton diversity and size?

Quantitative cytometry

Cytometry (*n.*): The counting and measuring of cells, especially the counting and analysis of cell size, morphology, and other characteristics.

Flow Cytometry

Conventional

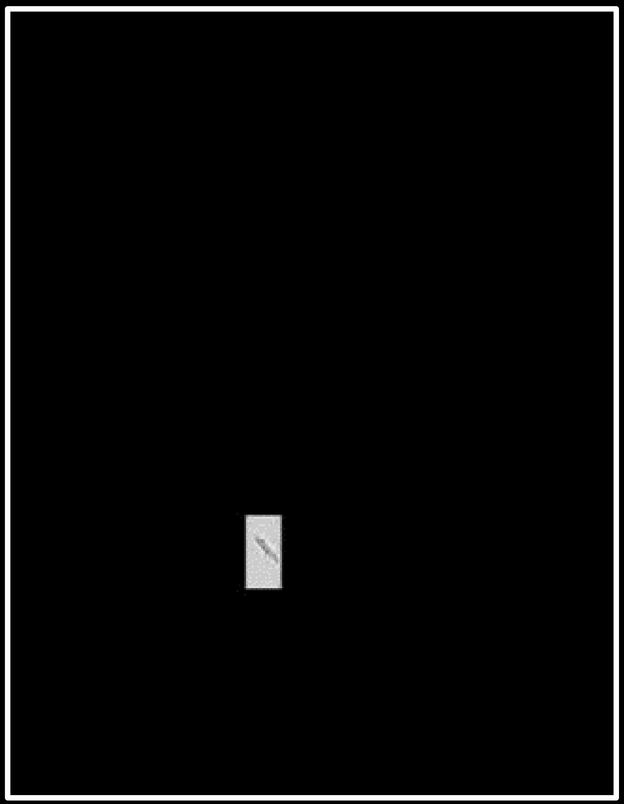


Single cell, typical measurements :

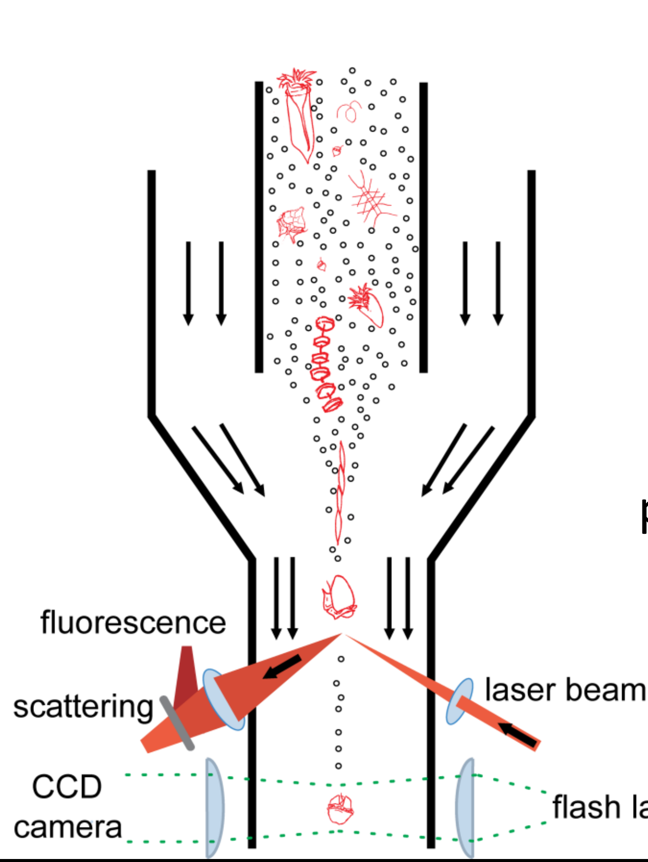
- Chlorophyll fluorescence
- Light scattering (forward, side angle)
- Phycoerythrin fluorescence

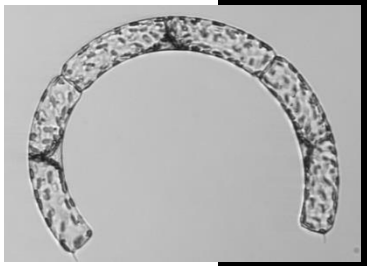
Sosik et al. 2014

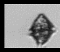
Flow Cytometry

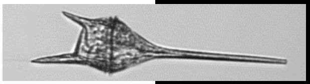


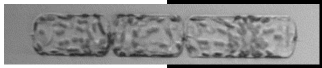
Imaging-in-Flow

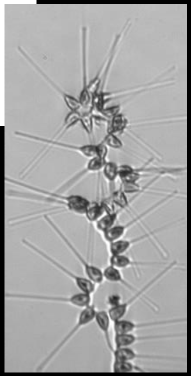












Same as conventional
Plus images
~ 1 µm resolution

Sosik et al. 2014

Automated and submersible flow cytometry

Circa 2001

FlowCytobot

Optimized for picoplankton

2017

Imaging FlowCytobot

Optimized for microplankton

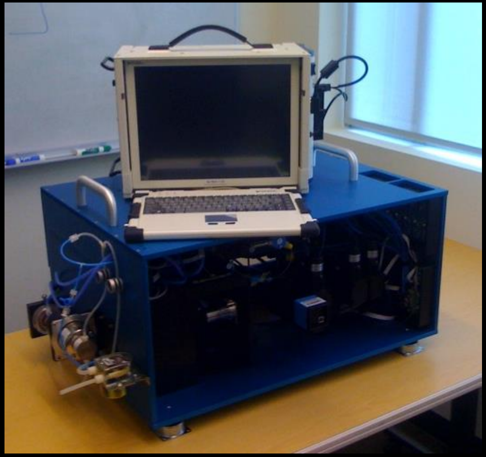
McLANE®
RESEARCH LABORATORIES, INC.

SPC

FlowCam

CytoSense

Jupiter microscope



SeaFlow

LISST-Holo

Automated and submersible flow cytometry

Circa 2001

FlowCytobot

Optimized for picoplankton

2017

McLANE RESEARCH LABORATORIES, INC.

Imaging FlowCytobot

Optimized for microplankton

→ Observational capabilities

Enumeration, identification, and cell sizing

Thousands of individual plankton

→ Extended deployments

Automated standard analysis, self-cleaning, and humidity sensing

> 6 months unattended

Taxonomic composition

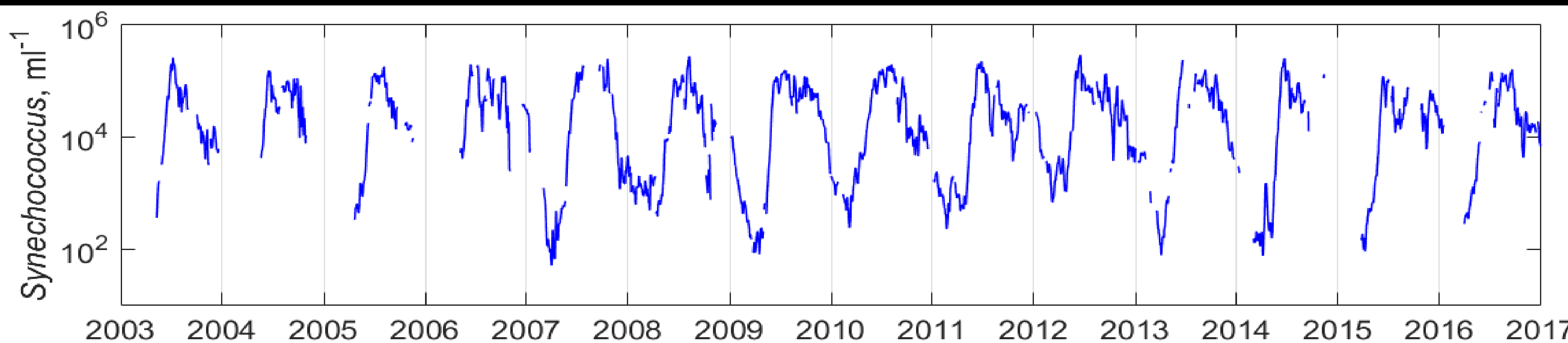
Circa 2001

FlowCytobot

Optimized for picoplankton

Martha's Vineyard Coastal Observatory

Hunter-Cevera et al. 2016



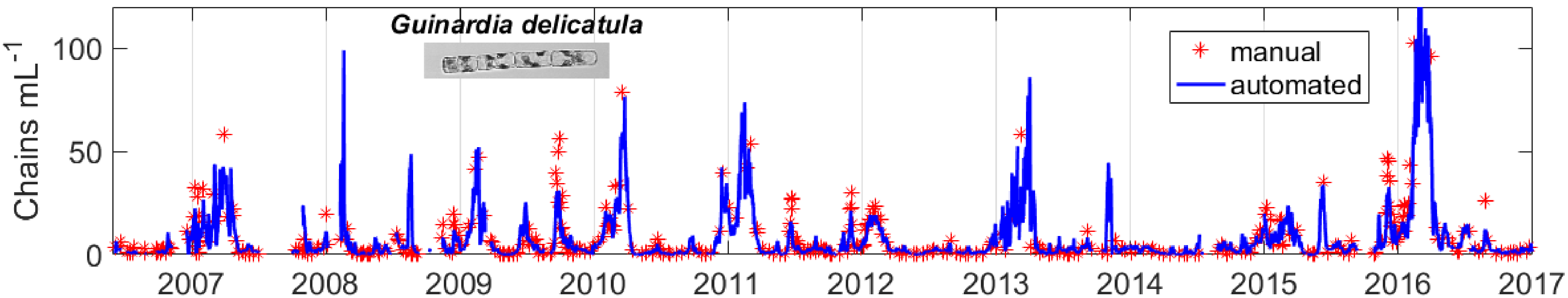
Taxon-specific
High resolution & Long duration

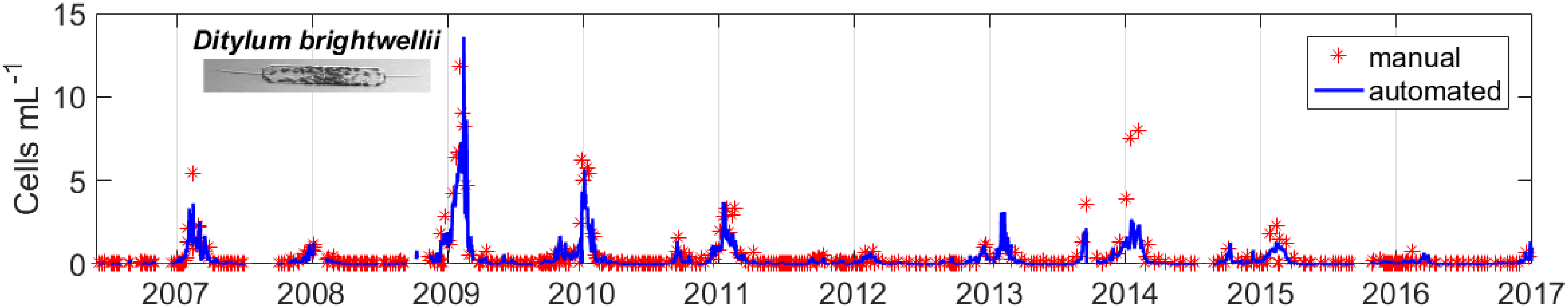
Taxonomic composition

Imaging FlowCytobot

Optimized for microplankton

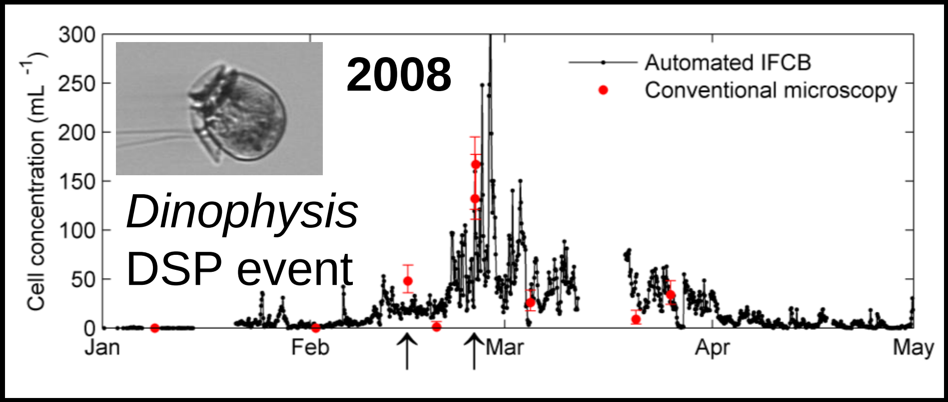
Martha's Vineyard Coastal Observatory





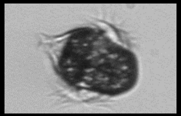
Harmful algal bloom – species-specific observations

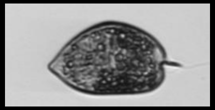
Imaging FlowCytobot

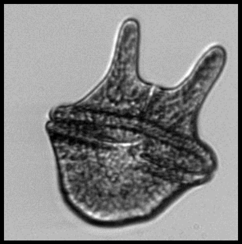


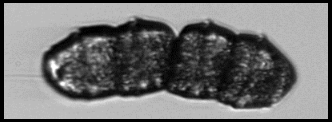
Gulf of Mexico

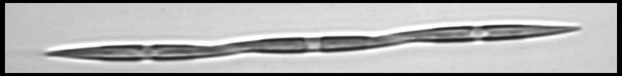
Campbell et al. 2010

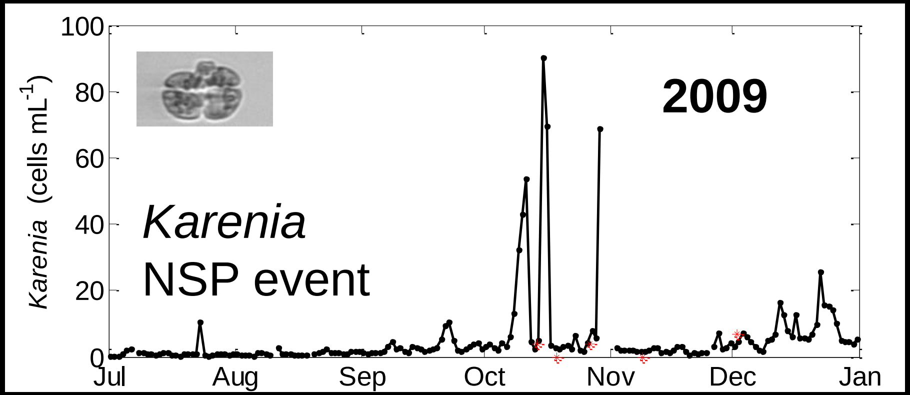




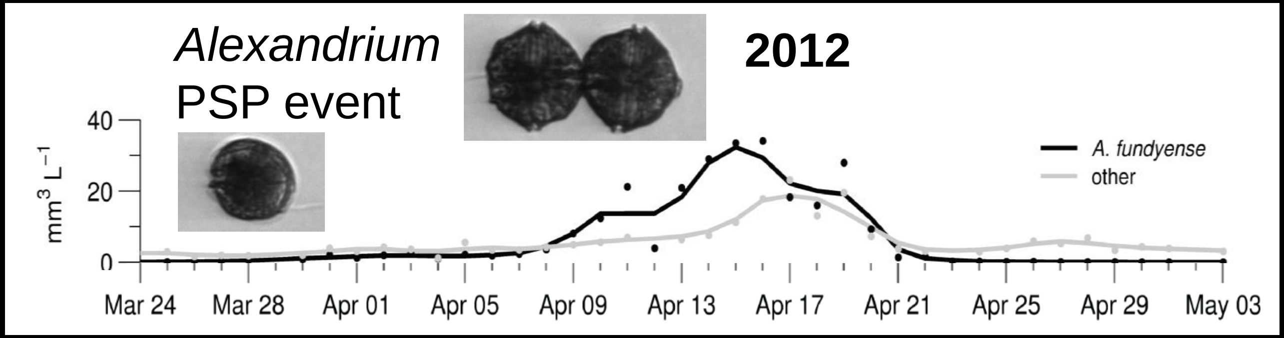








Campbell et al. 2013

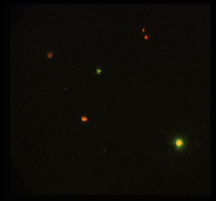


Nauset Estuary, MA

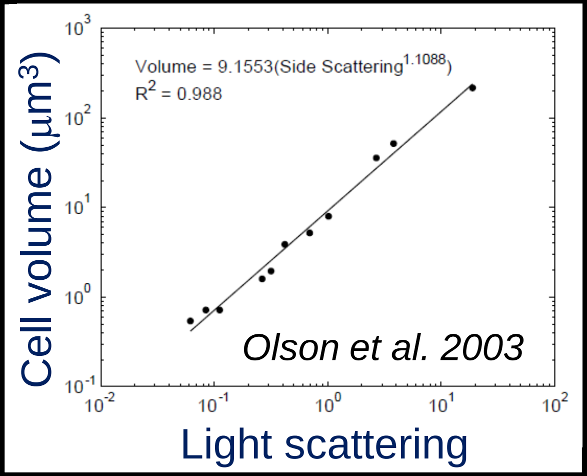
Brosnahan et al. 2015

Size and biomass budgets

Pico/nanoplankton



FlowCytobot



Cell volume from laser scattering

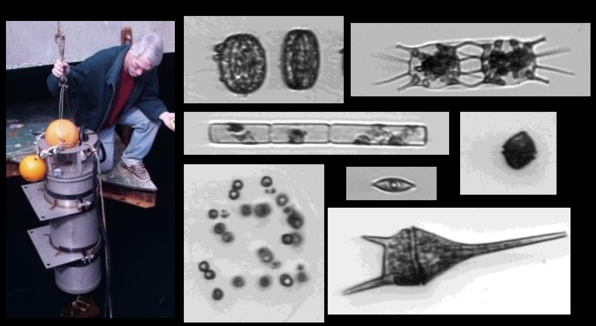
Cell carbon from cell volume

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

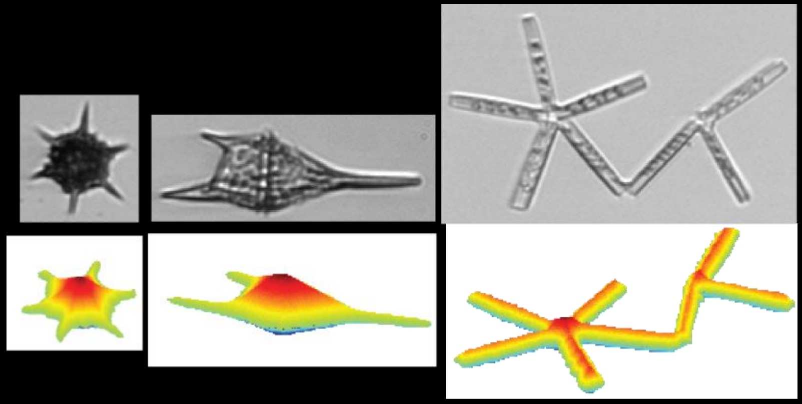
$$C_i = f(V_i)$$

e.g., Menden-Deuer & Lessard 2000

Nano/microplankton



Imaging FlowCytobot

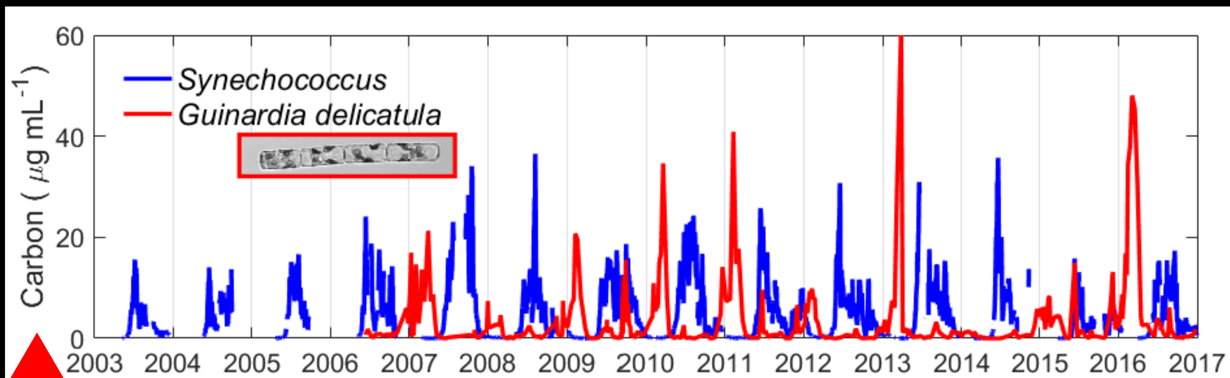


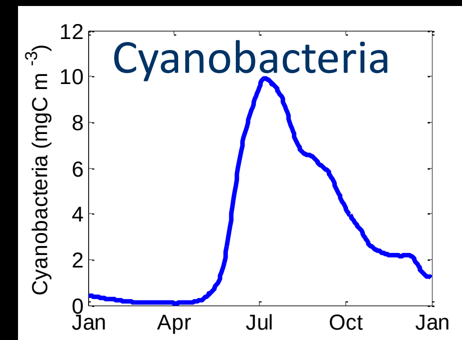
Cell volume from image analysis
"distance map" approach

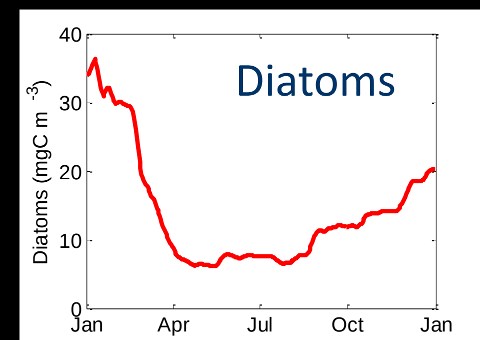
Sosik and Olson 2007
Moberg & Sosik 2012

Size and biomass budgets

Individual cells → Taxa → Communities

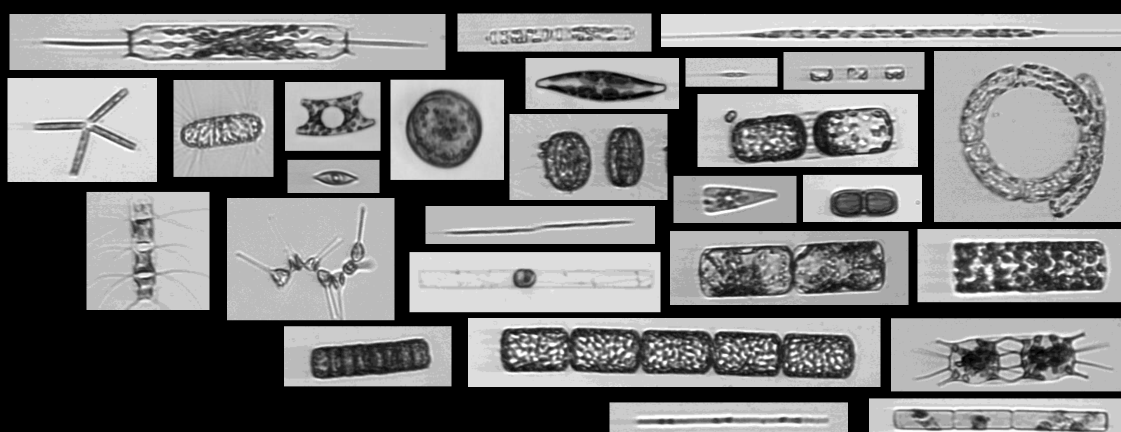






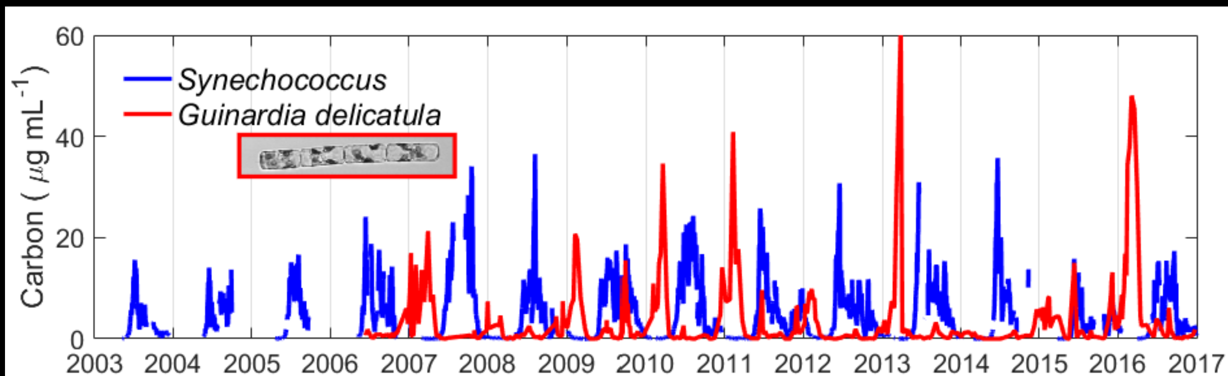
Carbon

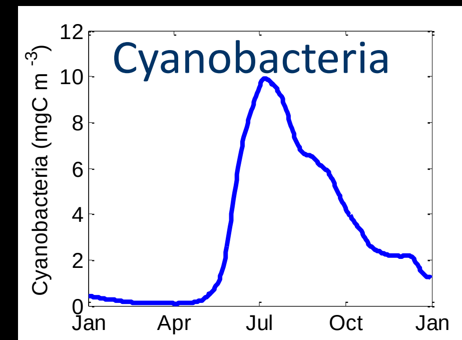
Many diatom species

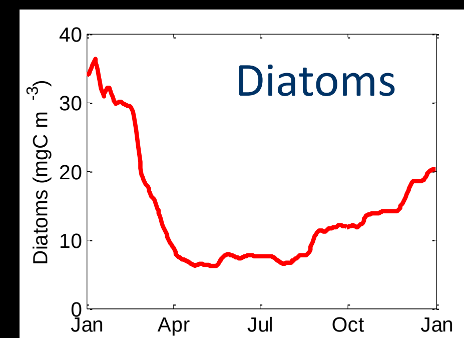


Size and biomass budgets

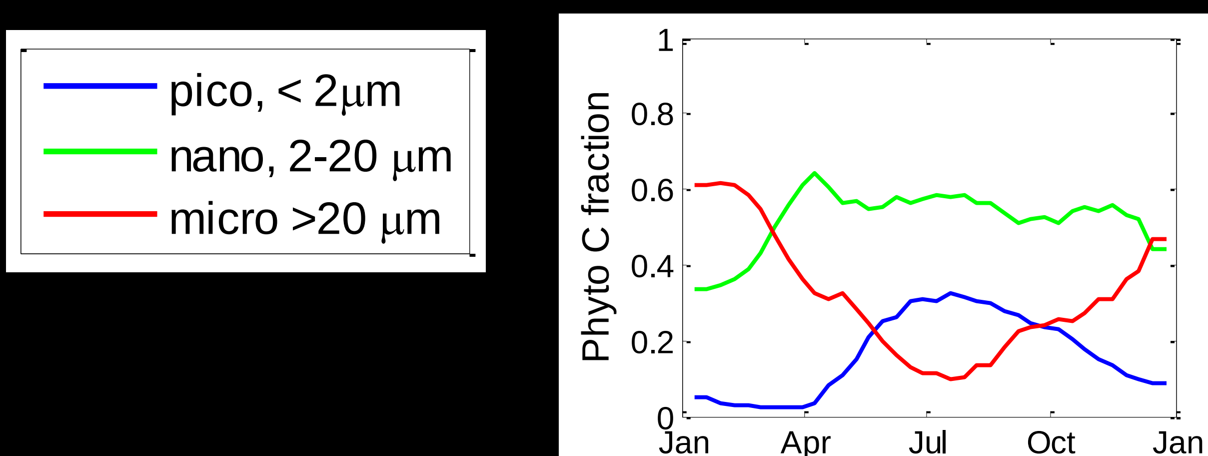
Individual cells → Taxa → Communities





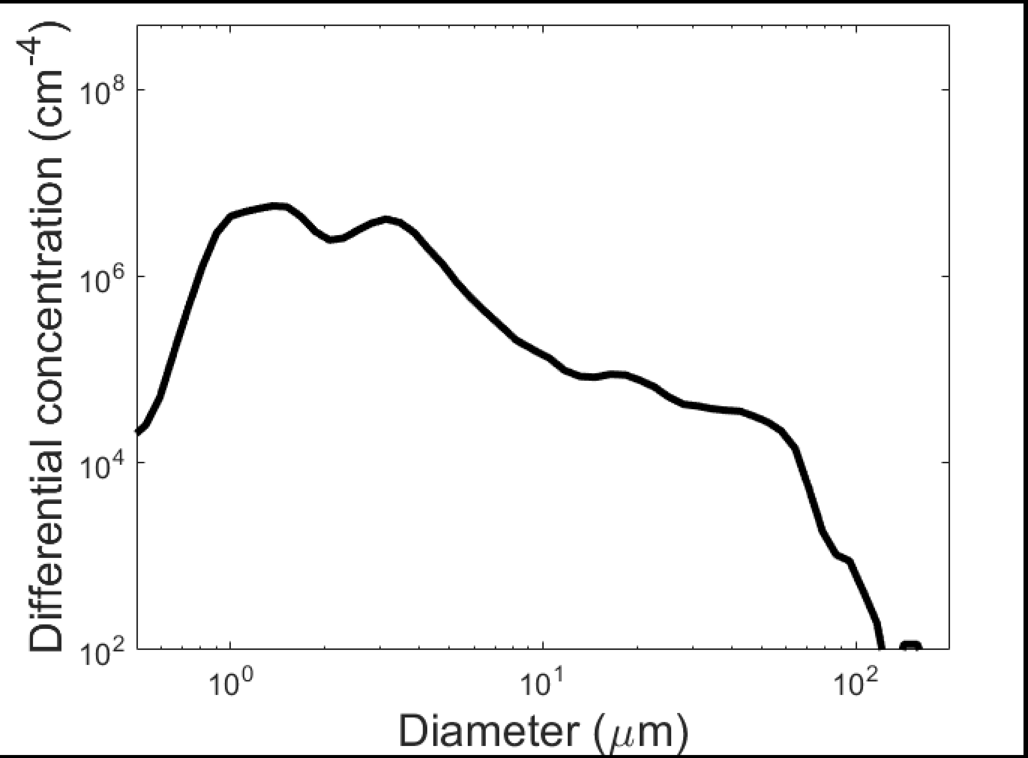


Individual cells → Size classes → Communities



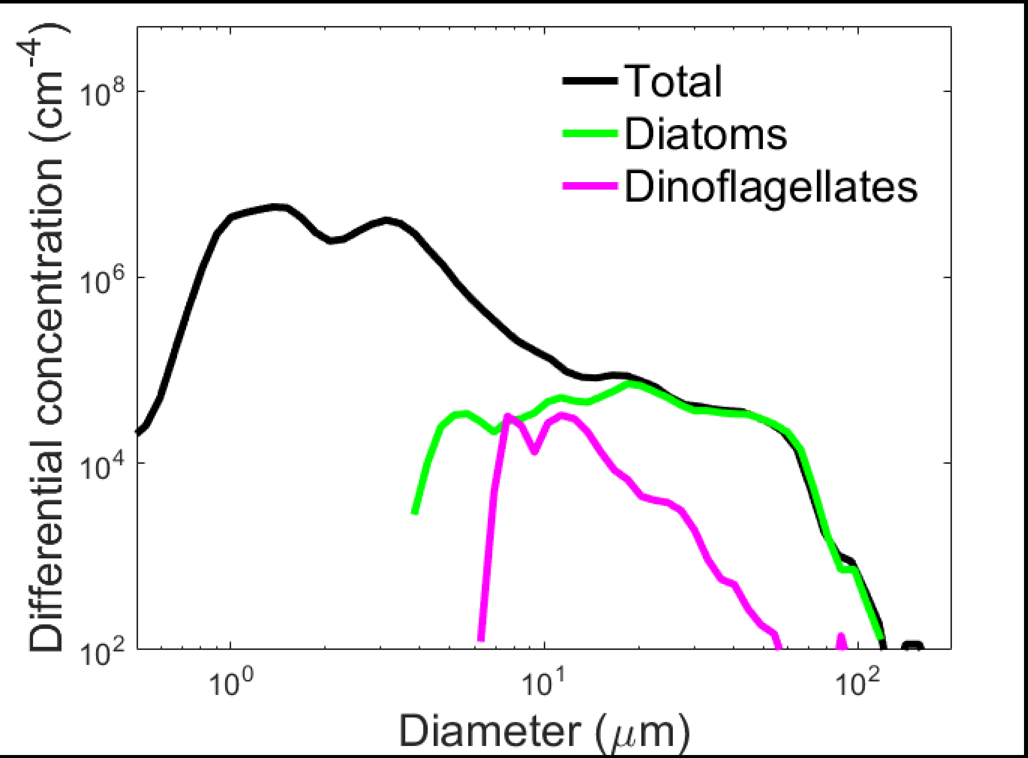
Phytoplankton size distributions

March



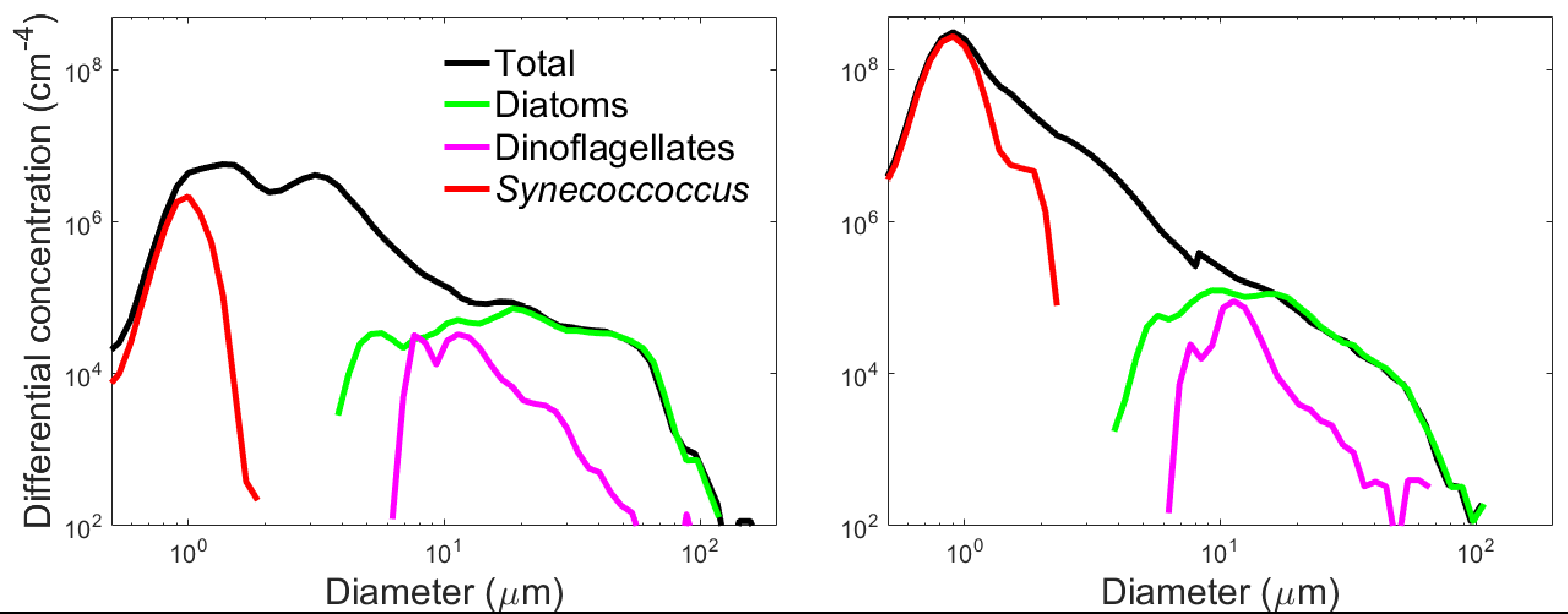
Phytoplankton size distributions

March

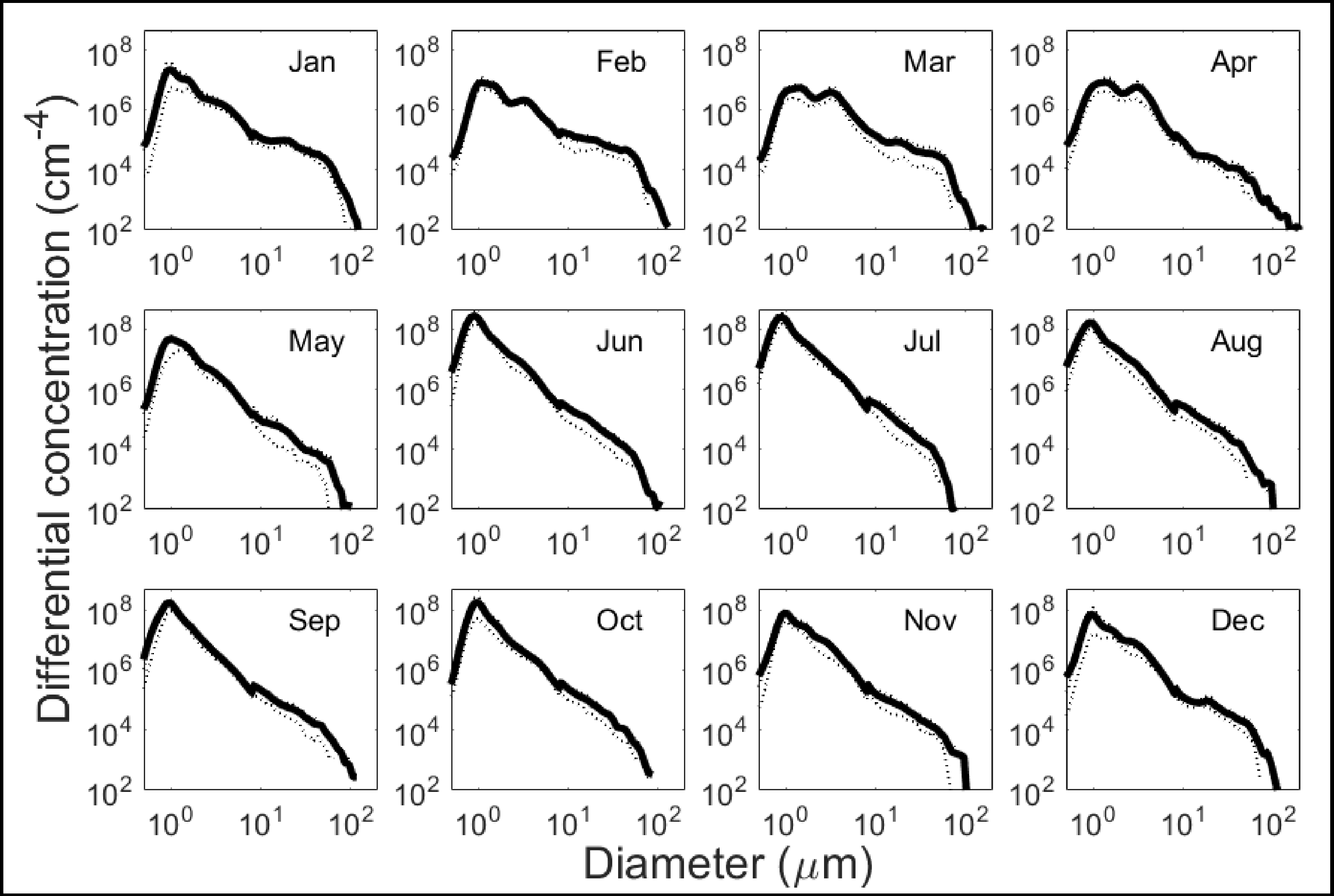


Phytoplankton size distributions

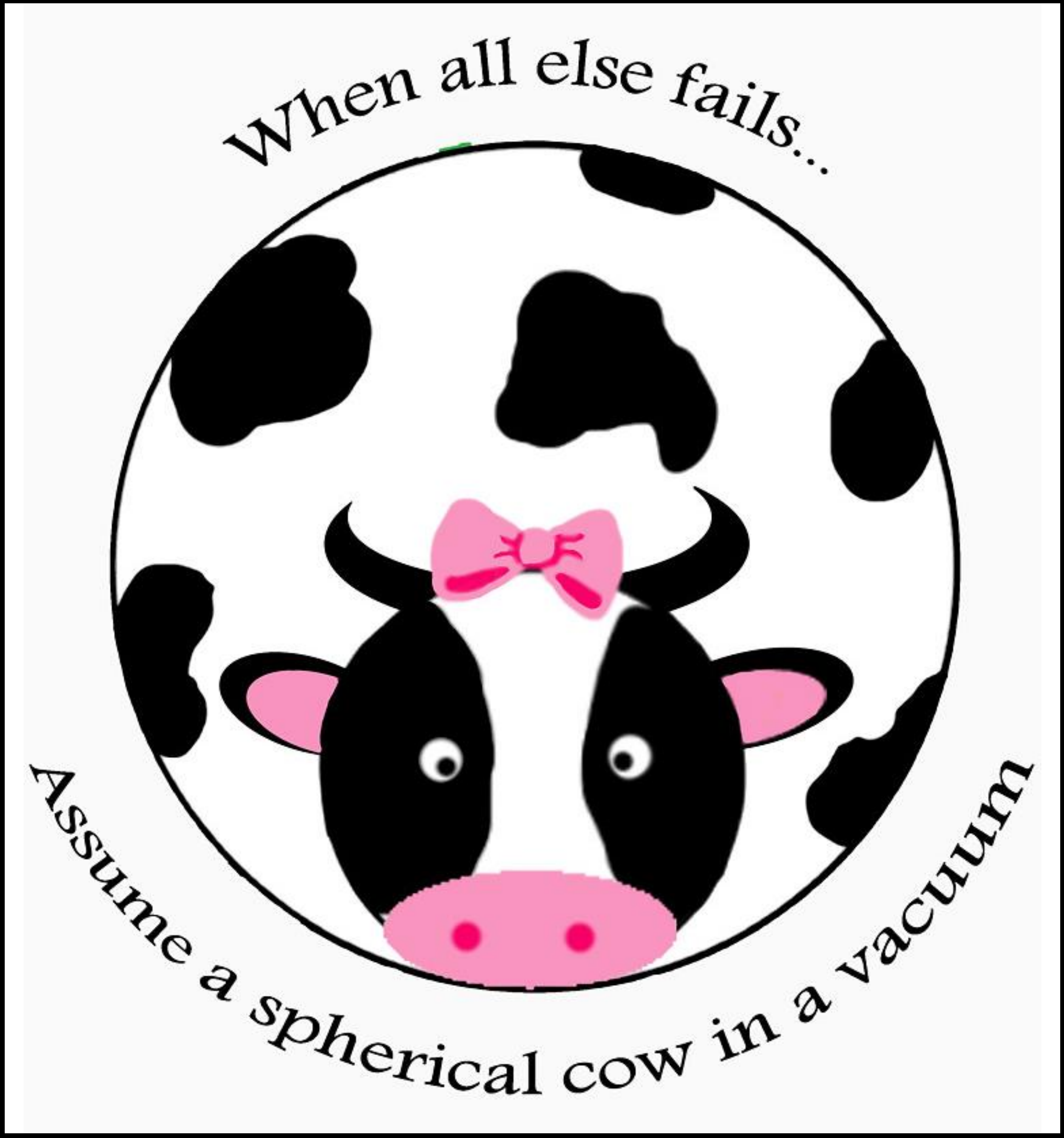
March June

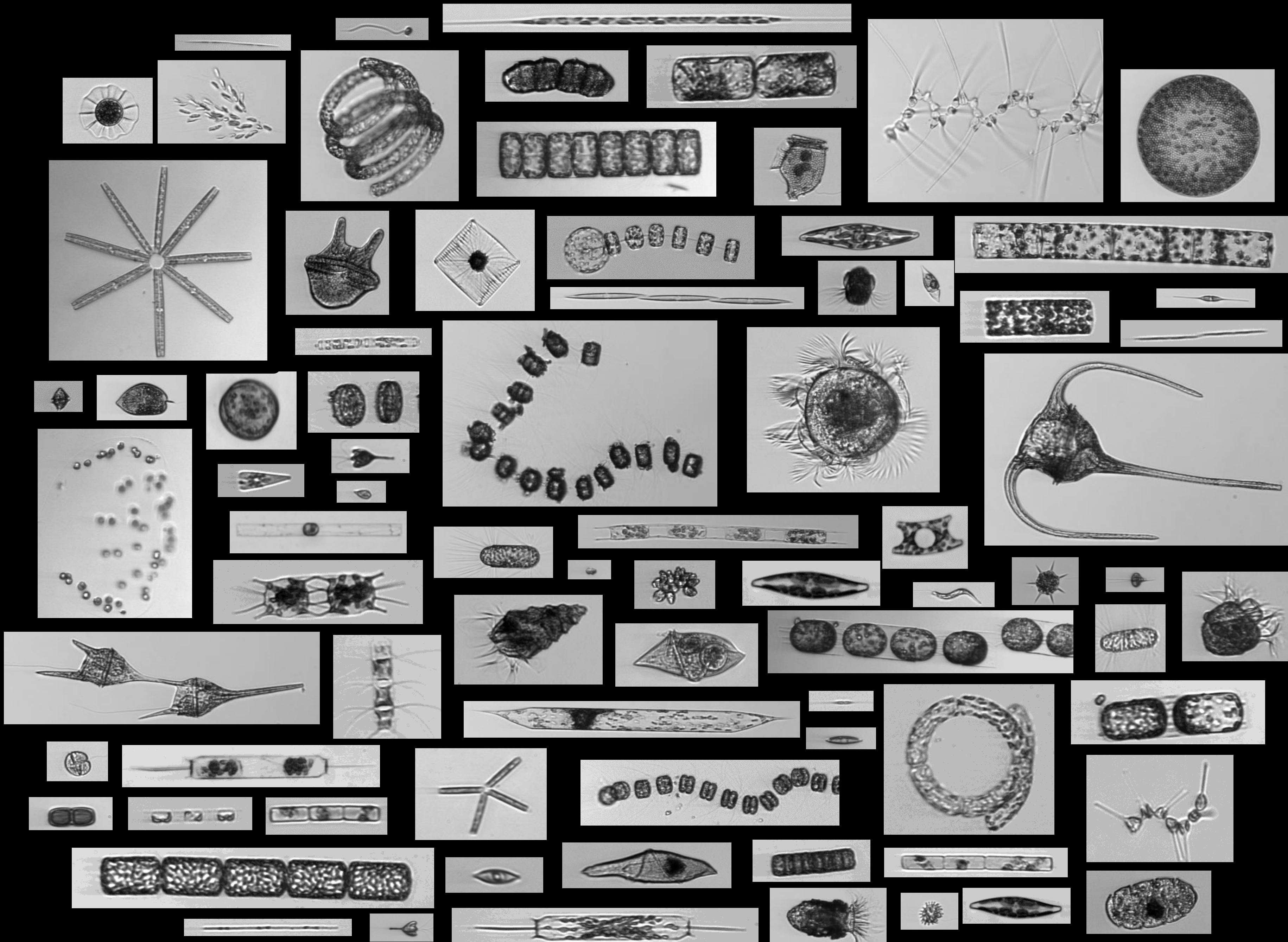


Phytoplankton size distributions



MVCO 2006-2016; monthly mean (——) 25th and 75th percentiles (····)


When all else fails...
Assume a spherical cow in a vacuum



Phytoplankton ≠ Spheres

Challenges for a baseline reset

Conceptual

- Size metrics
- Biomass metrics
- Taxonomic gaps

  N.B., many picoeukaryotes and small nanoplankton

Nuts and bolts and bits

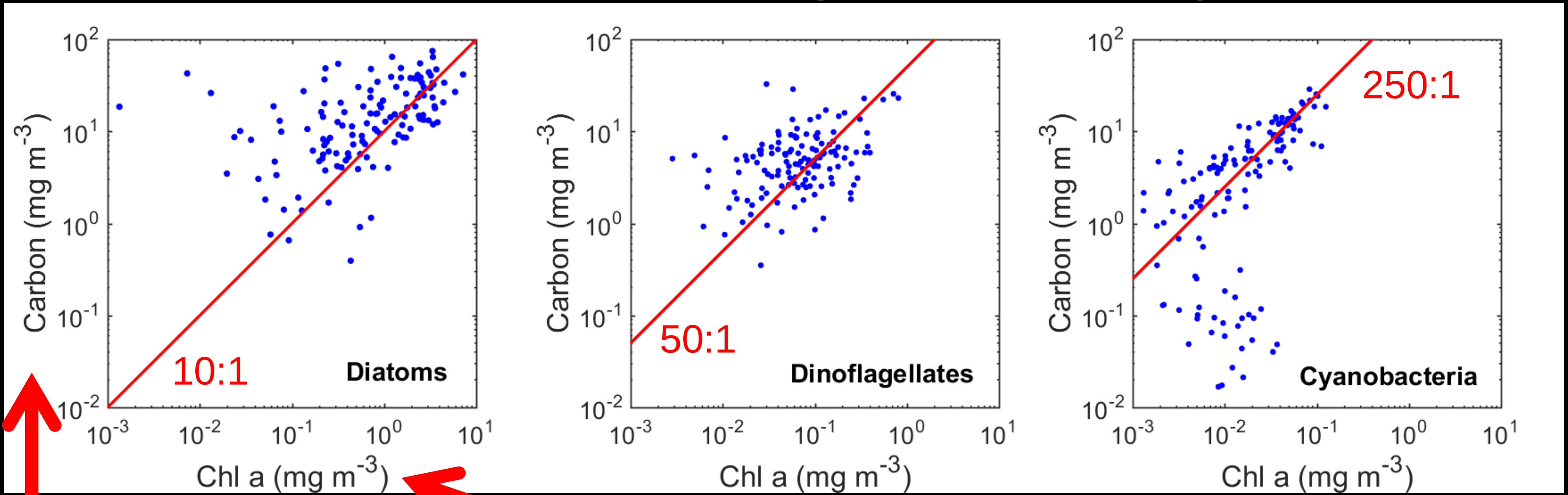
- Instrument development
- Operational quality control
- Analysis

Community building

- Data sets
- Annotated data
- Algorithms and workflows

Biomass estimation – comparing metrics

Diatoms Dinoflagellates Cyanobacteria



Flow Cytometry

CHEMTAX from HPLC

Implied Carbon : Chl variations very large

diatoms ~10

dinoflagellates ~50

cyanobacteria ~250

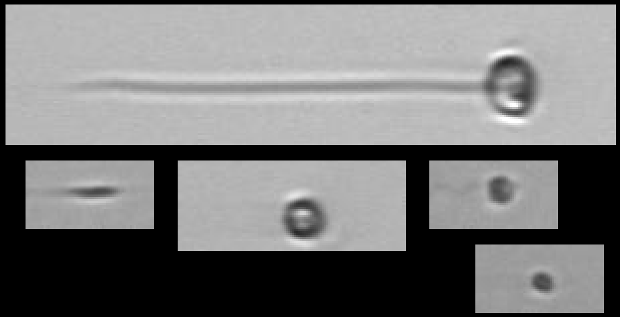
Chlorophyll $\neq$ Carbon

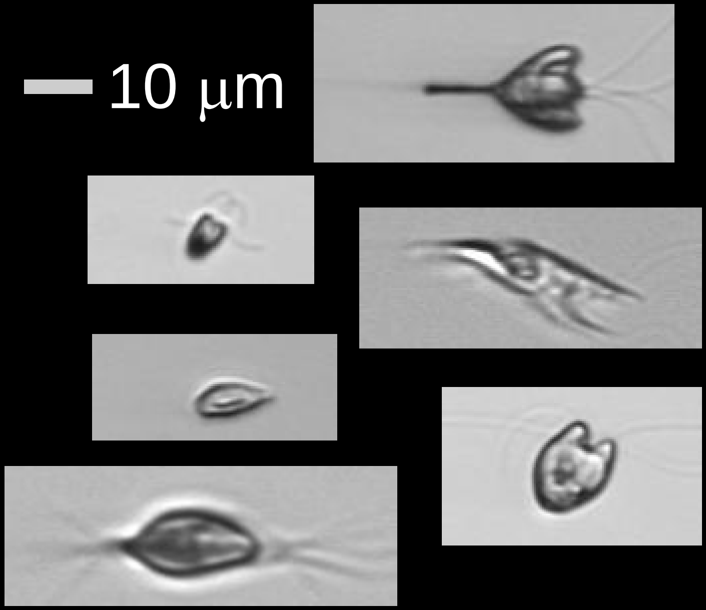
Challenges for a baseline reset

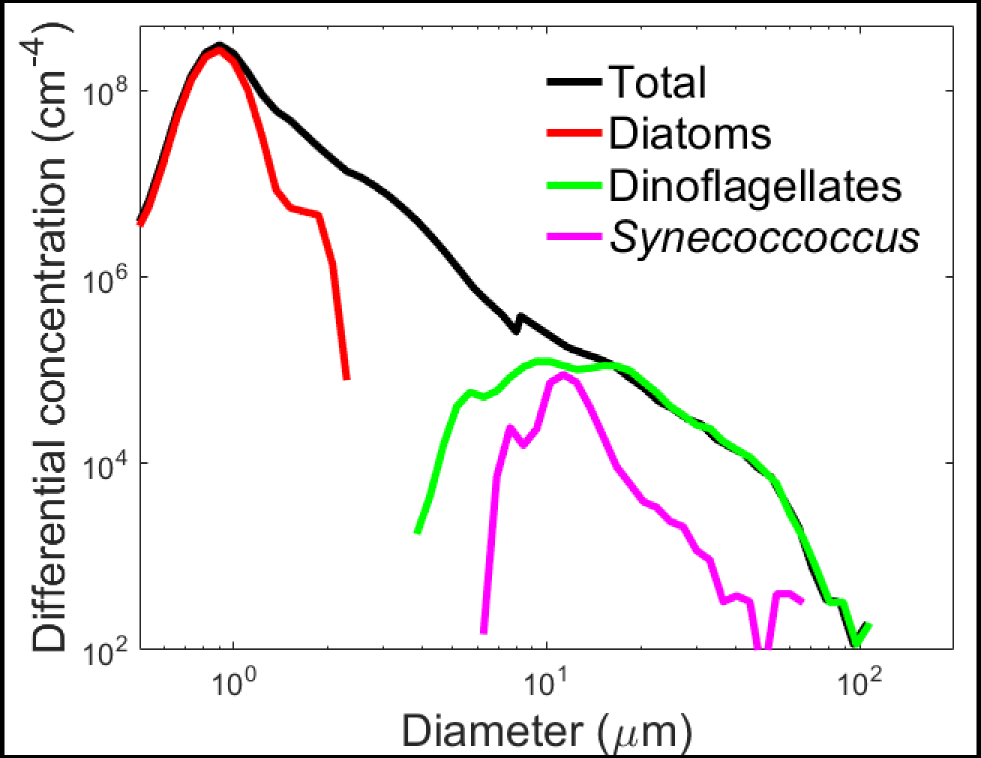
Conceptual

- Size metrics
- Biomass metrics
- Taxonomic gaps

N.B., many picoeukaryotes and small nanoplankton







Challenges for a baseline reset

Conceptual

- Size metrics
- Biomass metrics
- Taxonomic gaps
  - N.B., many picoeukaryotes and small nanoplankton

Nuts and bolts and bits

- Instrument development
- Operational quality control
- Analysis

Instrumentation targets

Smaller size

Lower power

Lower cost

➔ Accessibility & deployment modes

Sensitivity and dynamic range

e.g., the *Prochorococcus* challenge

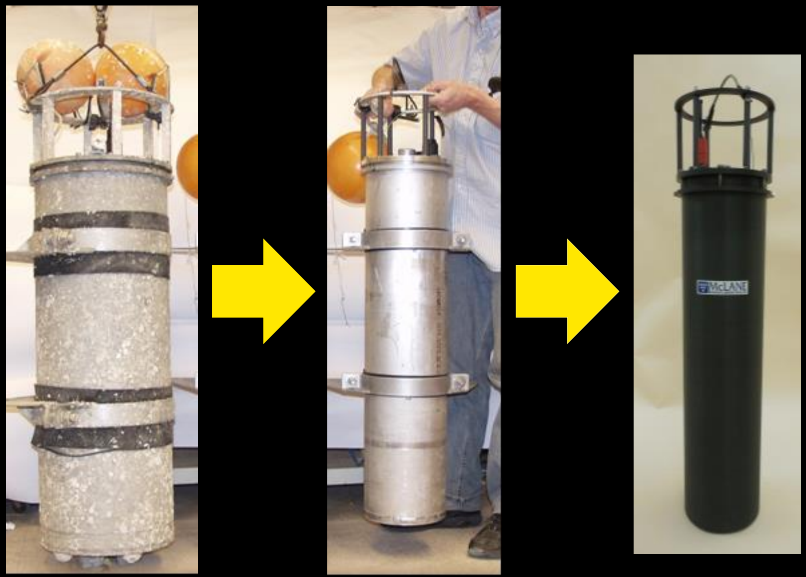
Sampling volume & resolution

Integrated anti-fouling & quality control

Enhanced onboard processing

…

ca. 2005 | 2012 | 2017



Original prototype | Optimized design | Commercial unit

Imaging FlowCytobot

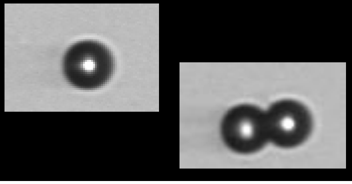
Good…but not good enough!

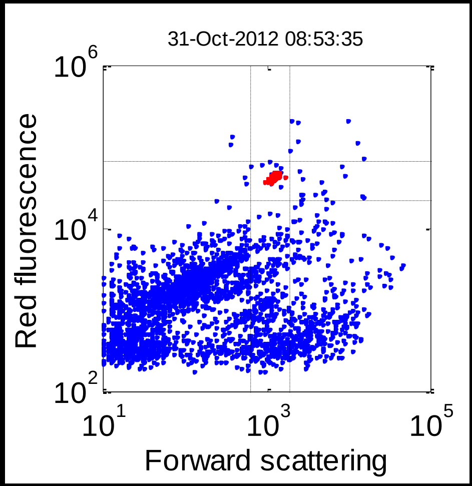
Integrated quality control

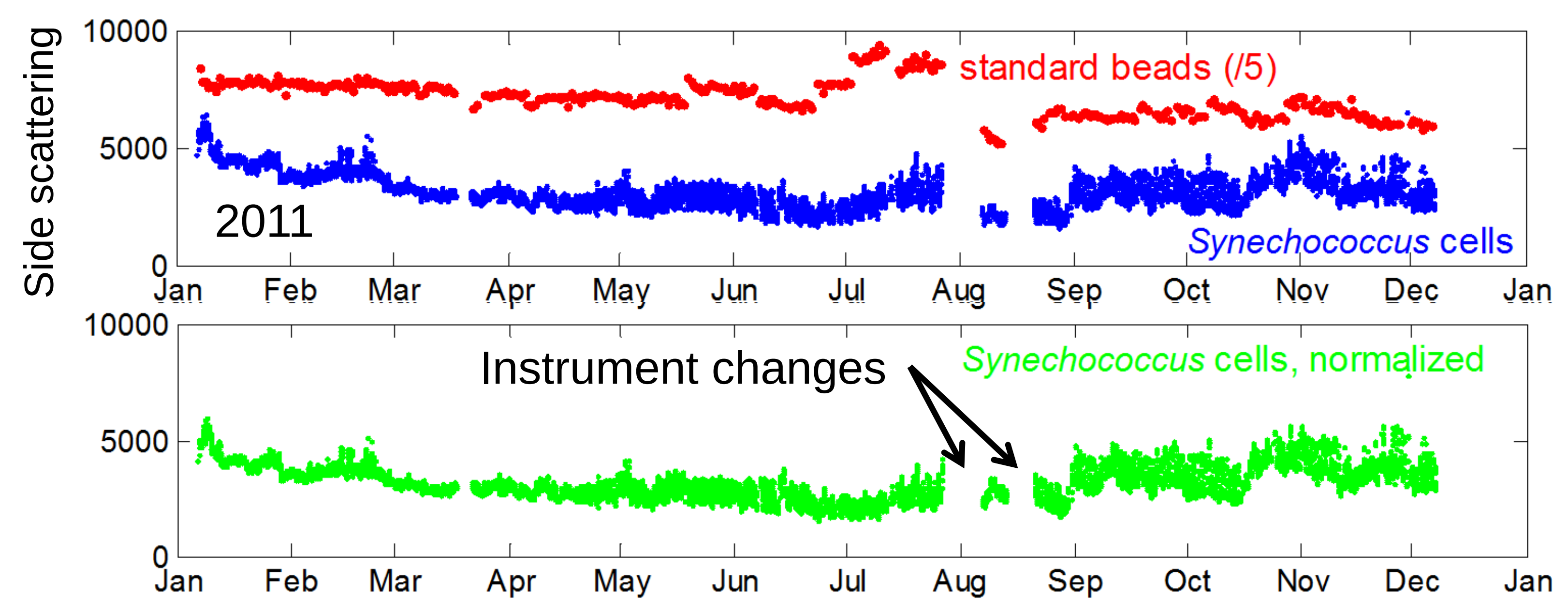
In situ standard analysis

Reservoir with fluorescent microsphere suspension

Automated analysis as “sample”



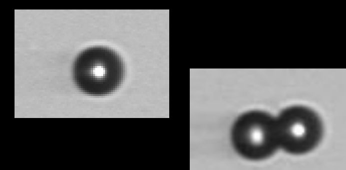


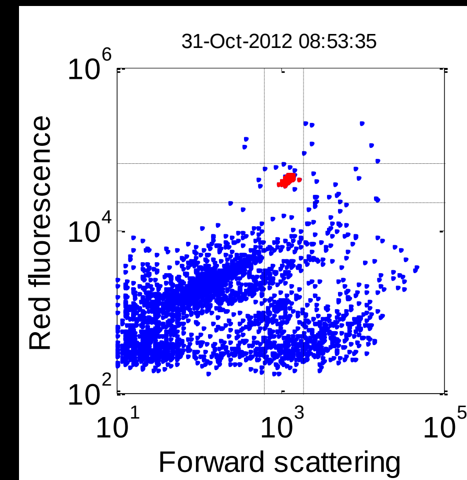


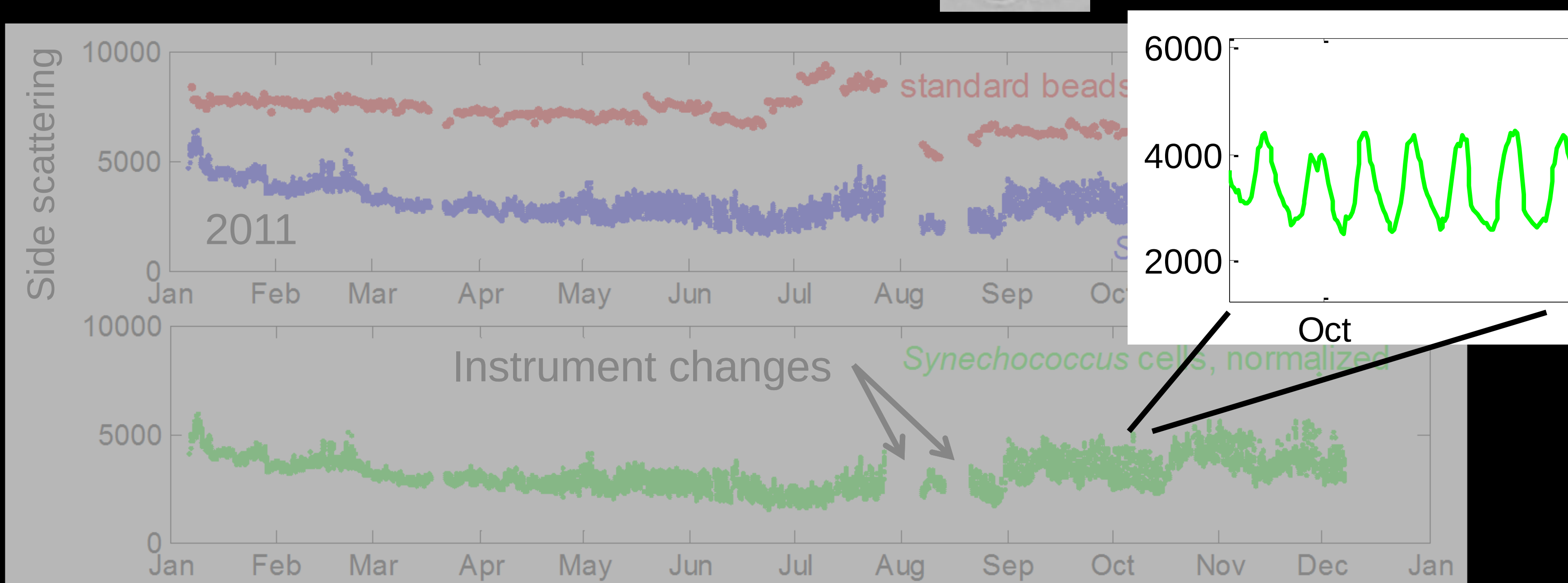
Integrated quality control

In situ standard analysis

- Reservoir with fluorescent microsphere suspension
- Automated analysis as “sample”

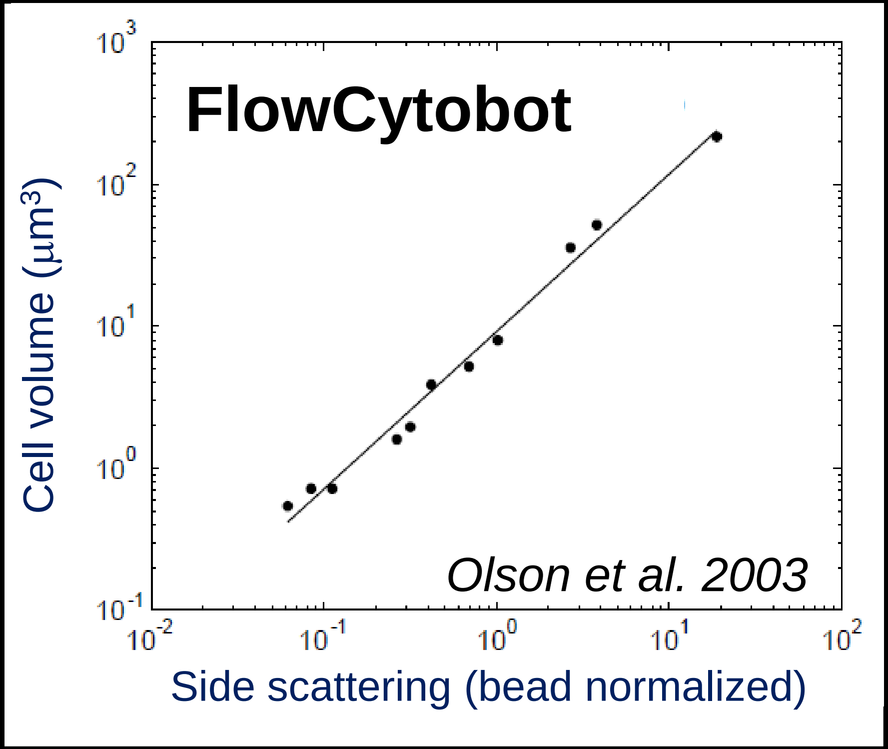


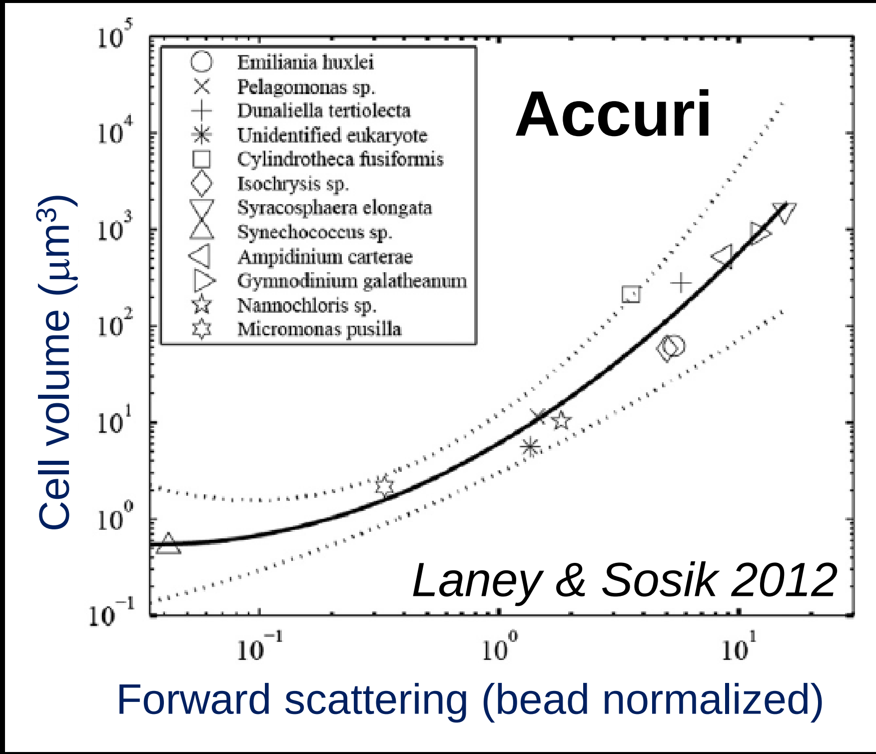




Data analysis and interpretation

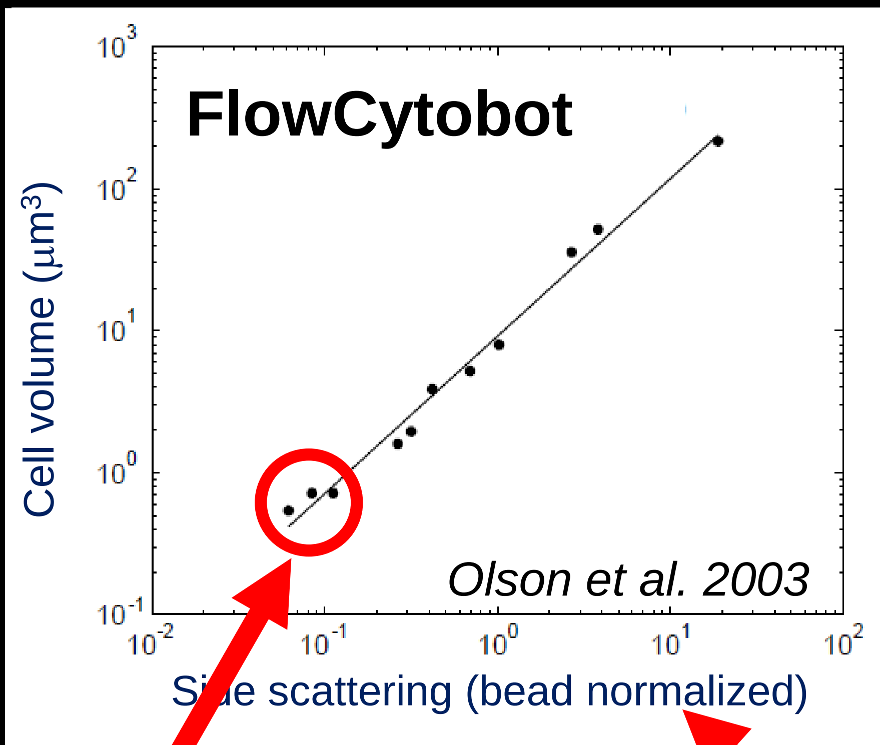
Cell volume calibration with culture analysis

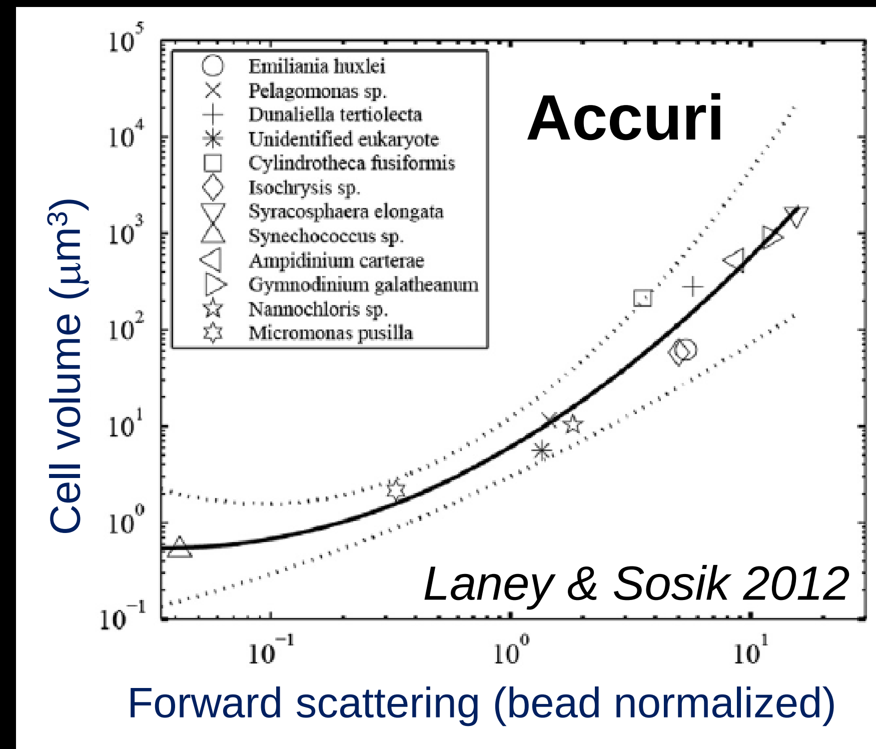




Data analysis and interpretation

Cell volume calibration with culture analysis





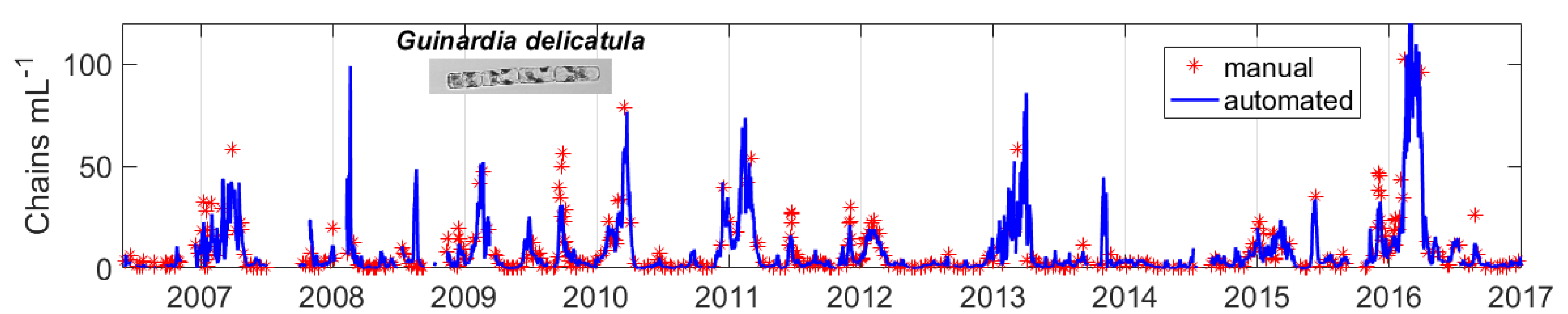
~1 µm cells

1 µm bead normalized

→Cells 10-fold lower scattering than beads of same size

Phytoplankton ≠ Plastic beads

Data analysis and interpretation



~800 million images of many species

Sosik and Olson 2007
Peacock et al. 2014

Image processing

Feature extraction

Classification, machine learning

Expert labeled training sets

Challenges for a baseline reset

Conceptual

- Size metrics
- Biomass metrics
- Taxonomic gaps

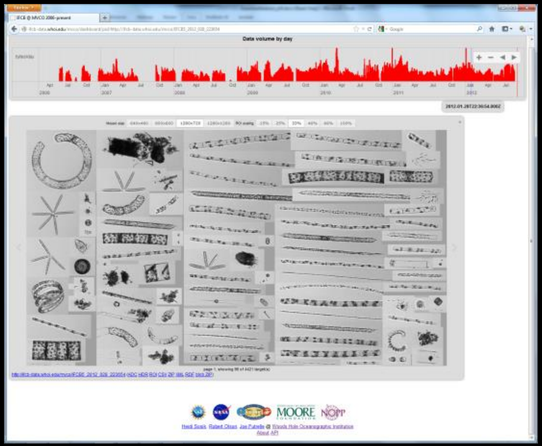
Nuts and bolts and bits

- Instrument development
- Operational quality control
- Analysis

Community building

- Data sets
- Annotated data
- Algorithms and workflows

Community building and shared resources



Shared data sets

http://ifcb-data.whoi.edu/

Shared annotations

training sets / expert taxonomy

http://dx.doi.org/10.1575/1912/7341

Shared algorithms and code repositories

https://github.com/hsosik/ifcb-analysis

https://github.com/joefutrelle/ifcb-dashboard

Standards for products,

with provenance to raw data and processing workflows

→ Promote reproducibility and support reprocessing

Acknowledgments

Rob Olson

Emily Brownlee
Taylor Crockford
Joe Futrelle
Kristen Hunter-Cevera
Ben Lambert

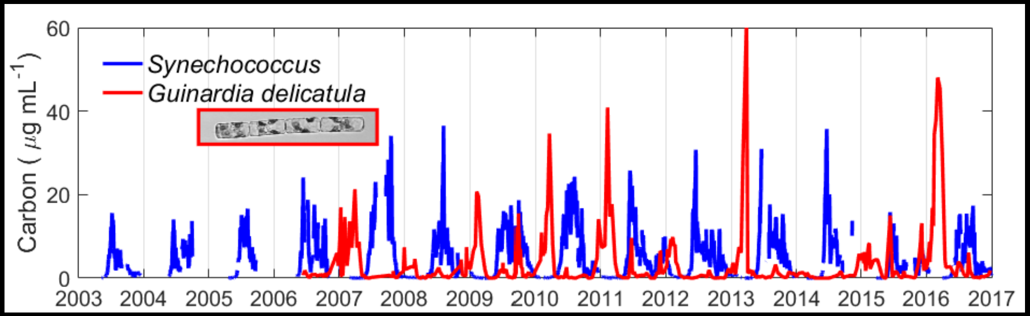
Emily Moberg
Emily Peacock
Alexi Shalapyonok

MVCO Operations Team

Challenges and prospects for a baseline reset

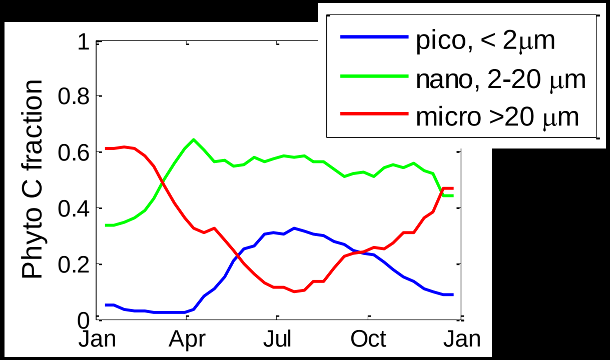
Conceptual

- Size metrics
- Biomass metrics
- Taxonomic gaps



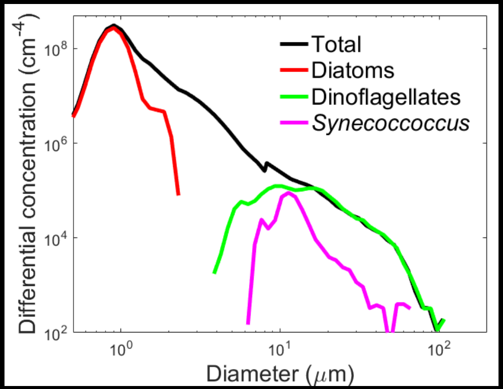
Nuts and bolts and bits

- Instrument development
- Operational quality control
- Analysis



Community building

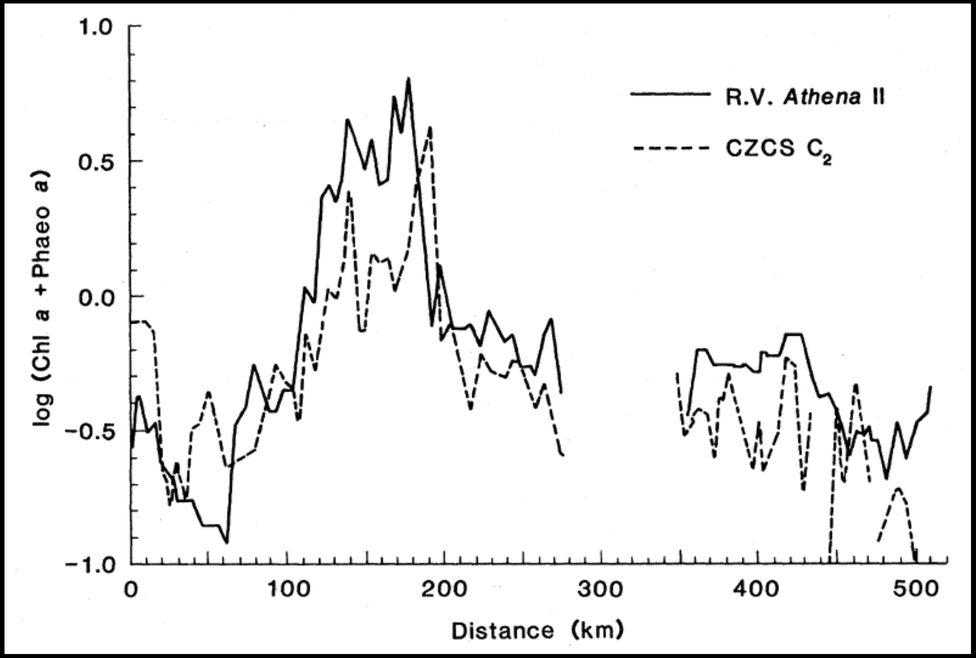
- Data sets
- Annotated data
- Algorithms and workflows



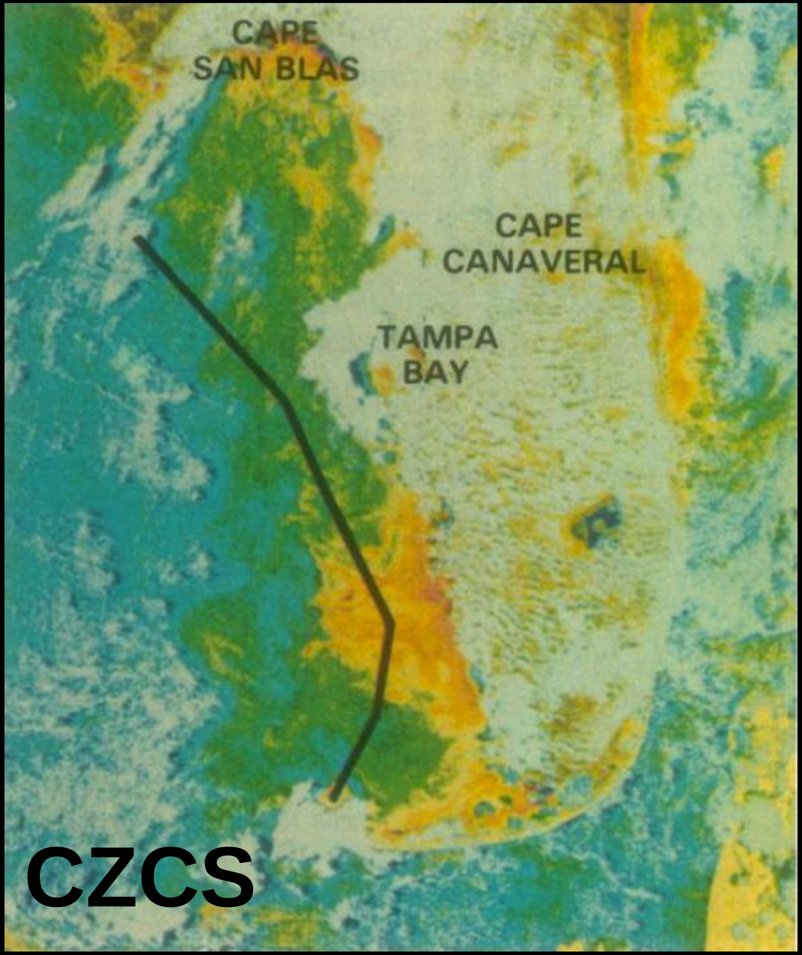
Current baseline for in situ phytoplankton measurements

Chlorophyll vs. distance along track

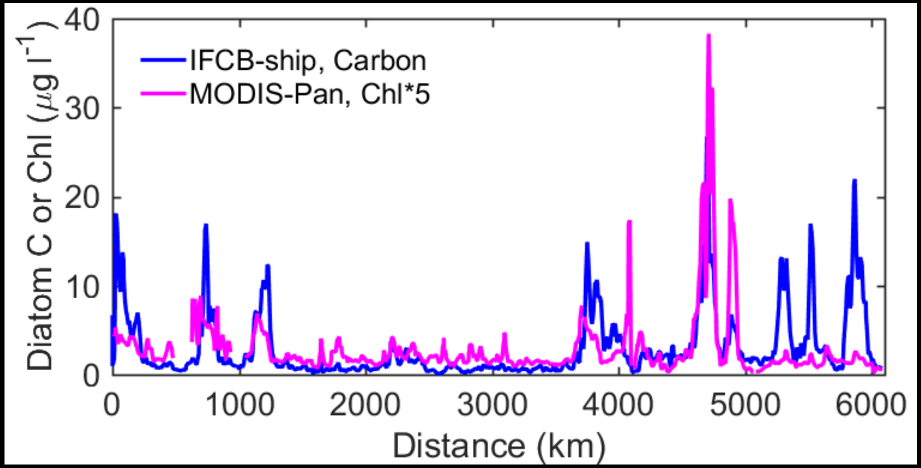
Gordon et al. 1980



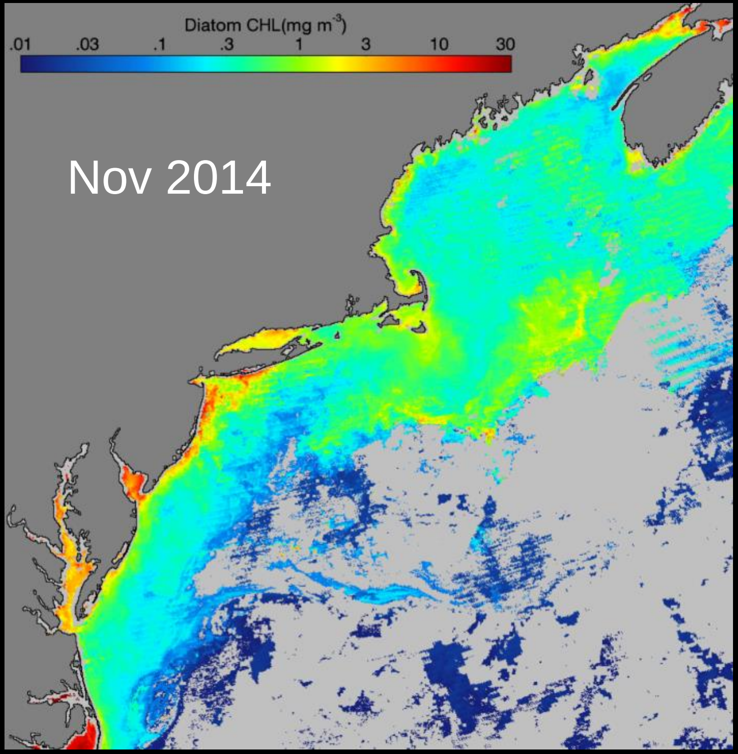
eBay

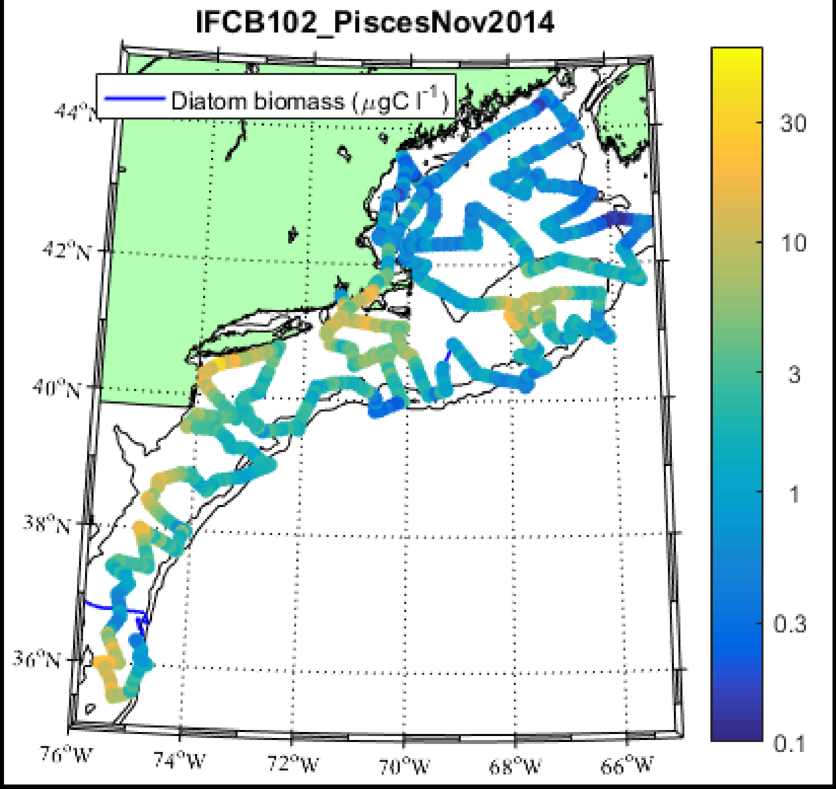


Baseline reset?



Diatom Biomass





MODIS PFT product

Kim Hyde