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

Lorenzen, 1966

Gieskes et al. 1978

Herman and Denman 1977
Current baseline for in situ phytoplankton measurements

Chlorophyll vs. distance along track  

Gordon et al. 1980

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

Pico- & Nano-plankton

Single cell, typical measurements:
Chlorophyll fluorescence
Light scattering (forward, side angle)
Phycoerythrin fluorescence

Sosik et al. 2014
Flow Cytometry

Imaging-in-Flow

Nano- & Micro-plankton

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
Automated and submersible flow cytometry

Circa 2001

FlowCytobot
Optimized for picoplankton

Observational capabilities
Enumeration, identification, and cell sizing
Thousands of individual plankton

→ Extended deployments
Automated standard analysis, self-cleaning, and humidity sensing
> 6 months unattended

Imaging FlowCytobot
Optimized for microplankton

2017
**Taxonomic composition**

**Circa 2001**

FlowCytobot

Optimized for picoplankton

Martha’s Vineyard Coastal Observatory

*Hunter-Cevera et al. 2016*

**Graph**

Graph showing the concentration of Synechococcus from 2003 to 2017.

*Taxon-specific High resolution & Long duration*
Taxonomic composition

Imaging
FlowCytobot

Optimized for microplankton

Martha’s Vineyard Coastal Observatory

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**Guinardia delicatula**

- *manual*
- *automated*

**Ditylum brightwellii**

- *manual*
- *automated*
Harmful algal bloom – species-specific observations

**Imaging FlowCytobot**

- **Gulf of Mexico**
  - *Campbell et al. 2010*

- **Karenia NSP event**
  - *Campbell et al. 2013*

- **Alexandrium PSP event**
  - *Brosnahan et al. 2015*

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**Dinophysis DSP event**

- 2008
- *Campbell et al. 2010*

**Karenia NSP event**

- 2009
- *Campbell et al. 2013*

**Alexandrium PSP event**

- 2012
- *Brosnahan et al. 2015*
Size and biomass budgets

**Pico/nanoplankton**

- 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**

- Cell volume from image analysis
  “distance map” approach

**Cells**

- Light scattering
- Imaging FlowCytobot

**FlowCytobot**

- Cell volume from laser scattering

**References**

- Olson et al. 2003
- Olsen et al. 2003
- Sosik and Olson 2007
- Moberg & Sosik 2012
Size and biomass budgets

Individual cells → Taxa → Communities

Many diatom species
Size and biomass budgets

Individual cells → Taxa → Communities

![Graph showing changes in phytoplankton carbon fraction over time]

Individual cells → Size classes → Communities

- **pico**, < 2 µm
- **nano**, 2-20 µm
- **micro**, >20 µm

![Graphs showing changes in cyanobacteria and diatoms carbon fractions over time]
Phytoplankton size distributions

March

![Graph showing phytoplankton size distributions in March](image)
Phytoplankton size distributions

March

![Graph showing phytoplankton size distributions in March. The graph plots differential concentration (cm$^{-4}$) against diameter (µm) with three lines representing total, diatoms, and dinoflagellates.](image)
Phytoplankton size distributions

March

June

Differential concentration (cm$^{-4}$)

Diameter ($\mu$m)

- Total
- Diatoms
- Dinoflagellates
- *Synecococcus*
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 nanoplanckton

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**
  - Implied Carbon: Chl variations very large
  - diatoms ~10

- **Dinoflagellates**
  - Implied Carbon: Chl variations very large
  - dinoflagellates ~50

- **Cyanobacteria**
  - Implied Carbon: Chl variations very large
  - cyanobacteria ~250

Flow Cytometry

CHEMTAX from HPLC
Chlorophyll ≠ Carbon
Challenges for a baseline reset

Conceptual

- Size metrics
- Biomass metrics
- Taxonomic gaps

N.B., many picoeukaryotes and small nanoplanクトon

10 μm

![Graph showing differential concentration vs. diameter with lines for Total, Diatoms, Dinoflagellates, and Synecococcus.](image)
Challenges for a baseline reset

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

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- Analysis
Instrumentation targets

Smaller size
Lower power
Lower cost

⇒ Accessibility & deployment modes

Sensitivity and dynamic range
e.g., the *Prochlorococcus* challenge

Sampling volume & resolution

Integrated anti-fouling & quality control

Enhanced onboard processing

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”

Instrument changes
Data analysis and interpretation

Cell volume calibration with culture analysis

FlowCytobot

Olson et al. 2003

Accuri

Laney & Sosik 2012

Forward scattering (bead normalized)

Side scattering (bead normalized)
Data analysis and interpretation

Cell volume calibration with culture analysis

FlowCytobot

Olson et al. 2003

Side scattering (bead normalized)

Cell volume ($\mu$m$^3$)

~1 $\mu$m cells

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

Accuri

Laney & Sosik 2012

Forward scattering (bead normalized)

1 $\mu$m bead normalized
Phytoplankton \neq 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
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Community building
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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
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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

CZCS

Cape San Blas

Cape Canaveral

Tampa Bay
Baseline reset?

Diatom Biomass

MODIS PFT product

Kim Hyde