Minimum Requirements for Lab/Field Measurements for PFT Algorithm Evaluation

Colleen Mouw

University of Rhode Island
Graduate School of Oceanography
cmouw@uri.edu

Photo credit: Chris Strait

Resources & Acknowledgements

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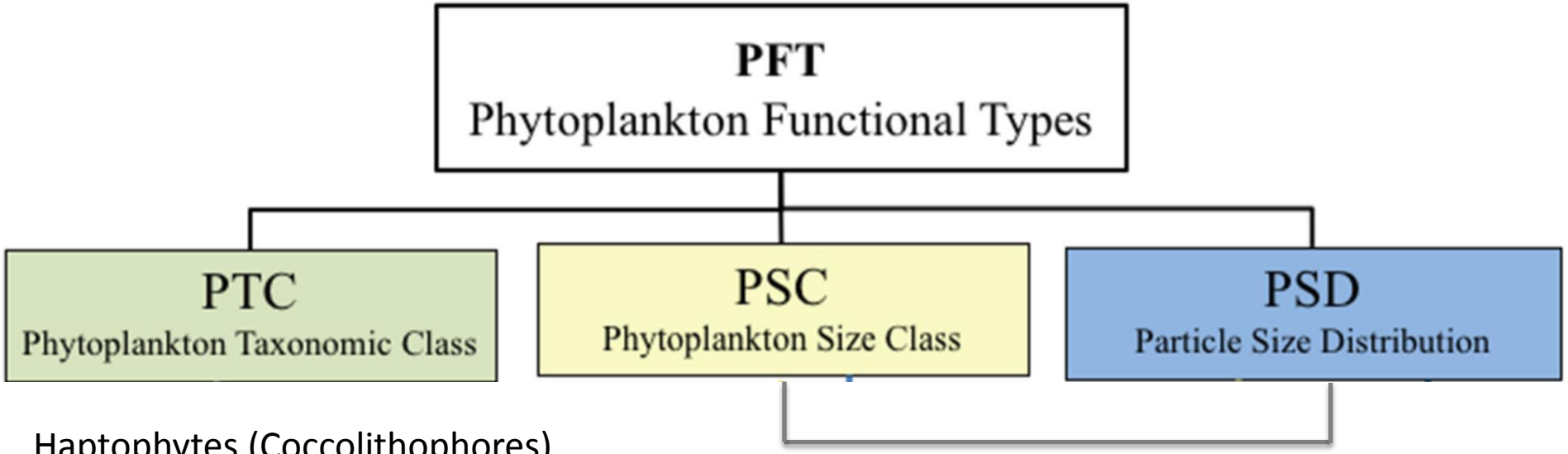
Satellite PFT Products



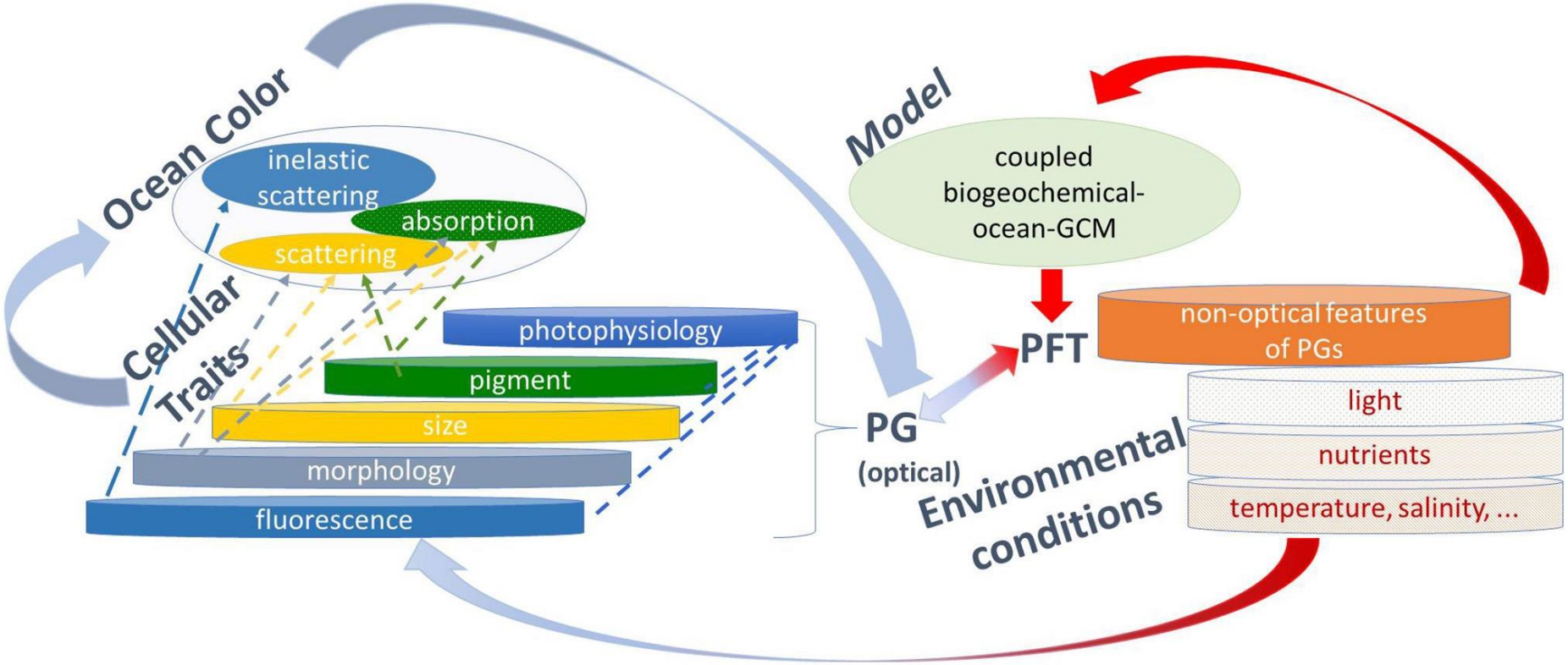
Haptophytes (Coccolithophores)
Dinoflagellates
Cyanobacteria (Pro/Syn)
Diatoms
Phaeocystis

Pico
Nano
Micro

Mouw et al. 2017

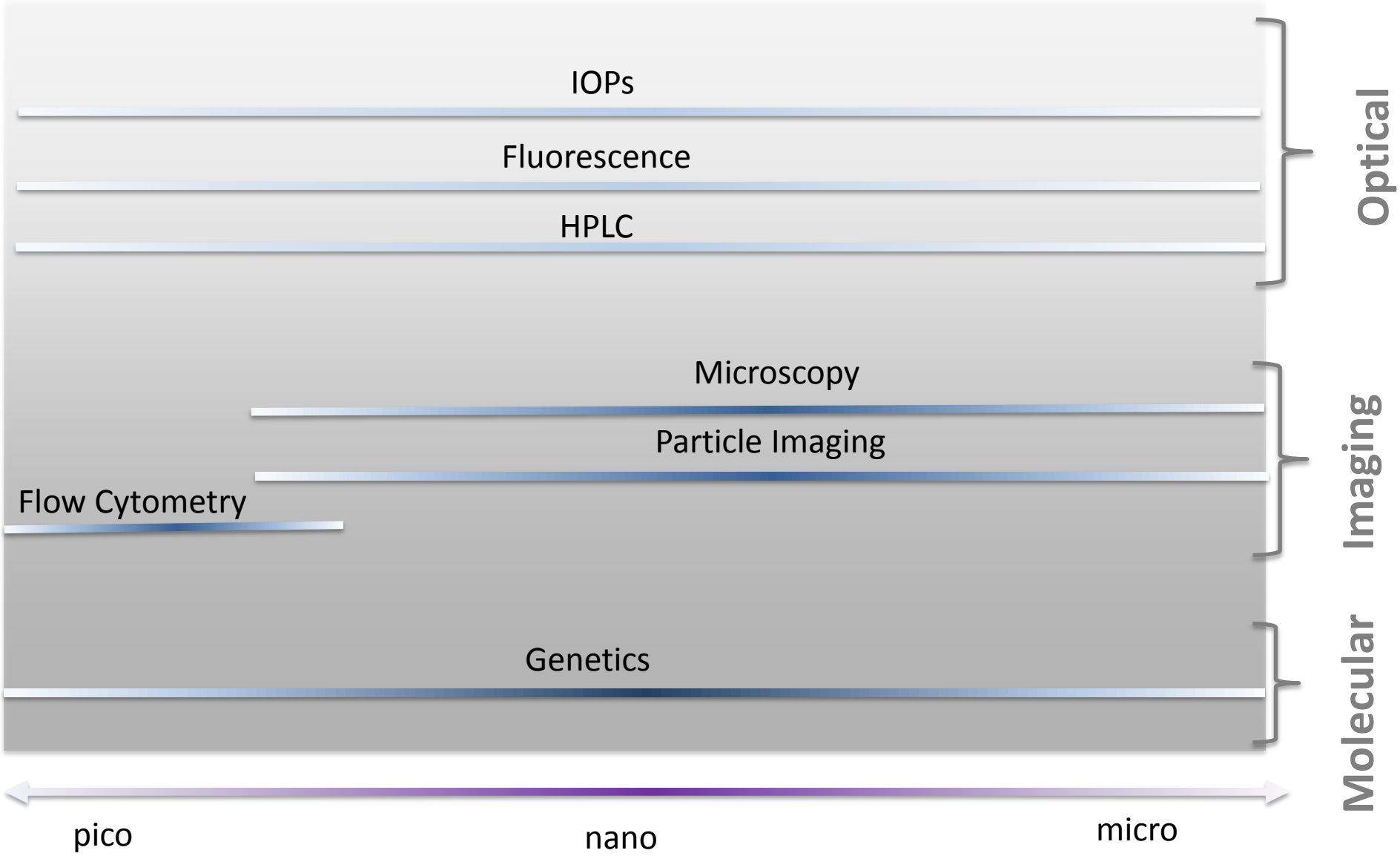
Motivation

- The majority of the existing PFT algorithms have been validated with HPLC pigment data.
- Despite its widely accepted use as an indicator of phytoplankton groups, the HPLC approach has not been well validated.
- Several other observation approaches are needed improve development and validation of satellite PFT algorithms.



Bracher et al. 2017

Range of Characterization & Level of Derivation from R_{rs}



Particle Imaging

Method	Advantages	Limitations	Relevance
Microscopy	• Only current method capable of identifying nearly all phytoplankton • Capital investment in equipment is low (at least for light microscopy) • Equipment maintenance is relatively straightforward	• Time consuming • Identification dependent on expert knowledge and subjective interpretation • Experts in phytoplankton taxonomy are increasingly rare • Assumptions required to convert to biovolume and biomass • Enumeration of small cells (picoplankton) requires the use of epifluorescence microscopy and identification of small cells (picoplankton) to species level is difficult • Sensitive to methods used to collect and concentrate cells and preservation techniques do not work equally well for all taxa	• Assumptions required to link cell counts to estimates of pigment biomass • Small sample sizes lead to large uncertainties in contribution of rare cells, which can be large contributors to biomass (due to large cell size) • Cell counts have to be supplemented with cell size information to estimate phytoplankton size structure
Flow Cytometry	• Automatic and fast • Picoplankton are readily observed • Imaging in flow provides access to microplankton • Potential for optically estimated cell size • In situ tools available	• Specialized instruments required to assess entire phytoplankton size range • Identification is often possible only to the level of certain phytoplankton groups • Instrumentation is expensive and delicate; requires expert user	• Assumptions required to link cell counts to pigment biomass • Cell abundance and cell size information can be converted to group-specific biovolume or carbon biomass

IOCCG, 2014

Particle Imaging – Instrumentation Differences

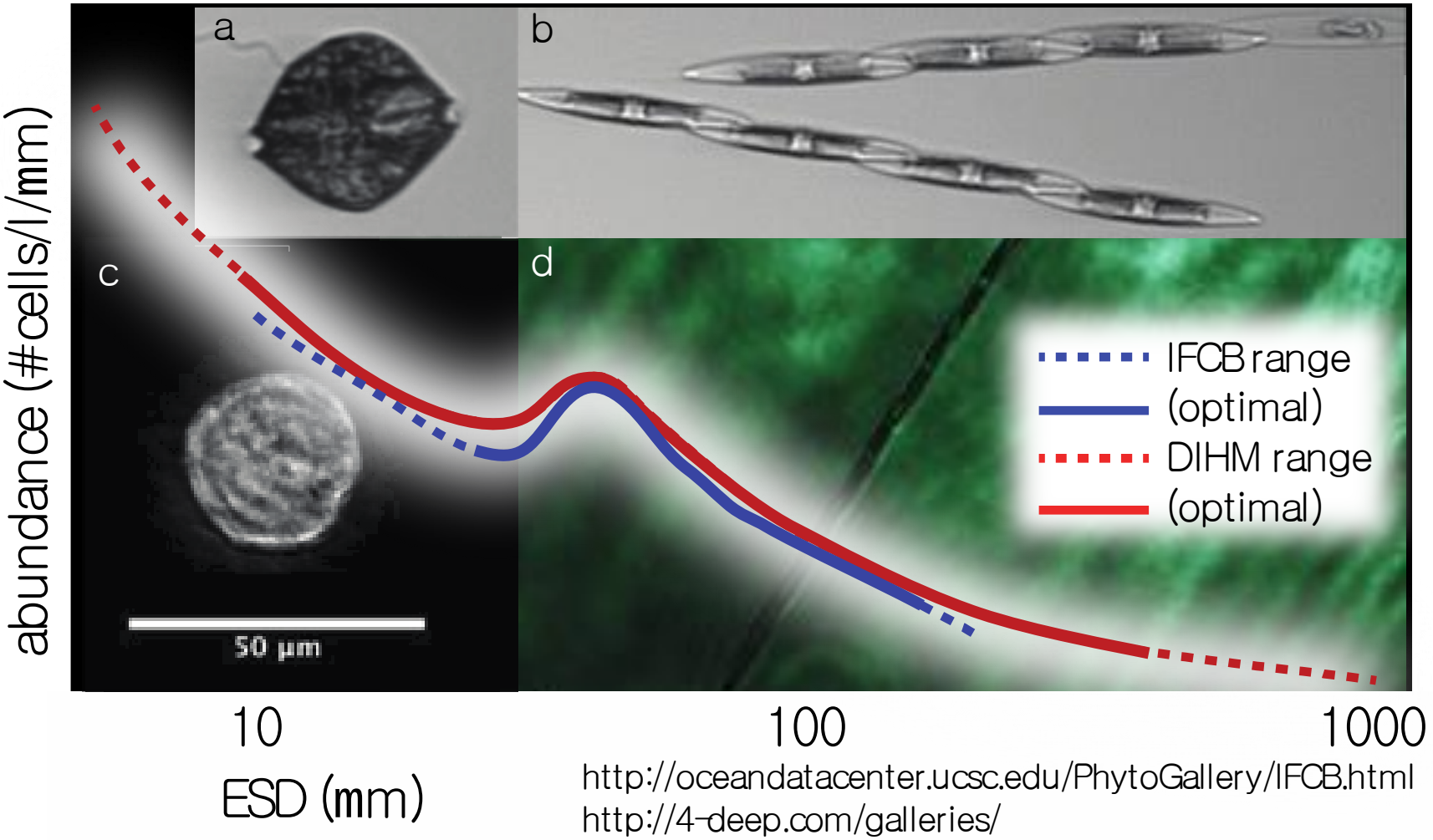


Figure: Courtesy of Melissa Omand

Optical

Method	Advantages	Limitations	Relevance
HPLC	• Automatic and precise • Basis of chemotaxonomy	• Few unambiguous marker pigments • Sensitive to assumptions about pigment ratios • Uncertainty caused by intra-group variability in pigment ratios (e.g., with growth conditions) • Expensive • Few experts and facilities, globally and comparison between laboratories confounded by differences in methodology (e.g., solvents, column materials) • No in situ tools	• Group-specific pigment biomass directly computed • Used extensively for development and validation of algorithms
IOPs	• Most methods are relatively simple and inexpensive • Many available tools, in situ and laboratory	• Assumptions required to convert between optical properties and biomass • Uncertainty in some methods caused by intra- and inter-group variability in optical properties (e.g., due to growth condition, taxonomy, etc.)	• Measurements directly linked to theoretical basis for remote sensing of phytoplankton
Successive Filtration	• Relatively simple	• Cell breakage and filter clogging can lead to inaccuracies • Practical constraints impose limits on the number of size classes that can be measured • Time consuming • No taxonomic information	• Most direct assessment of size-based biomass

IOCCG, 2014

Optical

Method	Advantages	Limitations	Relevance
Fluorescence Ex/Em Spectra	• Simple and inexpensive method for extracted chlorophyll concentration • In vivo excitation and emission spectra useful for some group-specific assessment • Rapid and easy • In situ tools available	• Interpretation of *in vivo* fluorescence signal is complex and dependent on taxonomy and physiology • Assumptions required to convert *in vivo* signals to biomass • Uncertainty caused by intra-group variability in pigments and associated fluorescence	• Basis of most total phytoplankton biomass assessments • Used in active-passive remote sensing to detect phytoplankton types from laser-based remote sensing from aircraft • Solar-induced chlorophyll fluorescence is amenable to remote sensing

IOCCG, 2014

-Omics

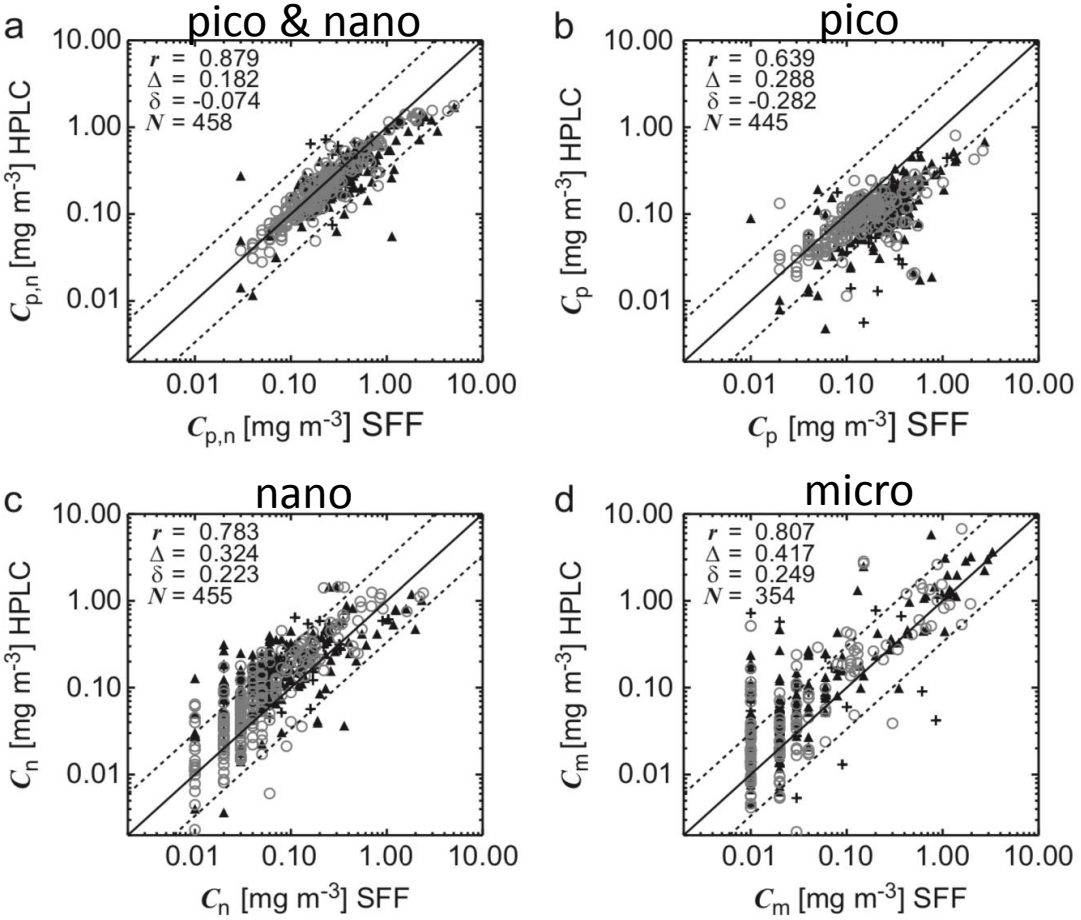
Method	Advantages	Limitations	Relevance
Molecular Methods	• Taxa can be targeted with high degree of specificity • Particular functions can be targeted directly • In situ tools emerging	• Only a few probes currently available • Method development and testing time consuming • Relatively expensive and requires specialized equipment • Assumptions required to convert to biomass or size structure	• Largely untested

IOCCG, 2014

Efforts to Compare Methods

Comparison of size fractionation and sized estimated with HPLC pigments

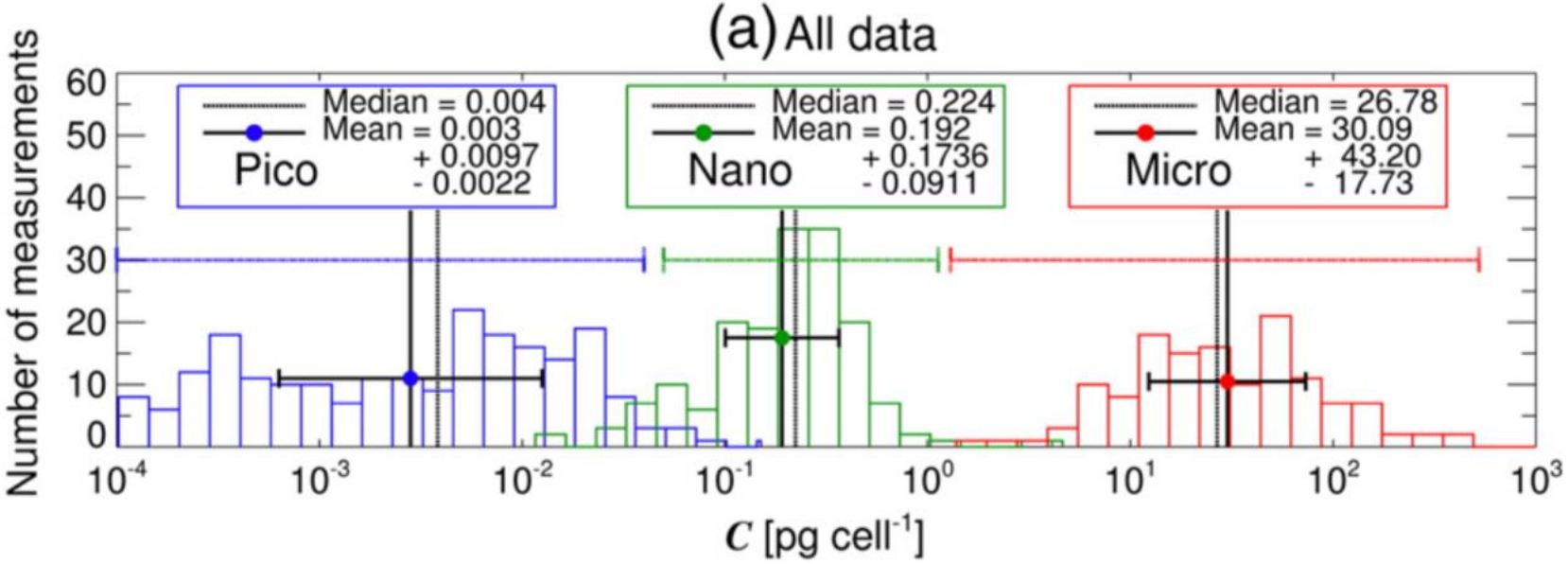
- HPLC data explained between 40 - 88% of the variability in the size fractionated data.
- Significant biases between the methods:
  - HPLC overestimating nanoplankton chlorophyll and underestimating picoplankton chlorophyll compared with size fractionation.
- Uncertainty in both HPLC and size fractionation data makes it difficult to ascertain which is more reliable.



Brewin et al. 2014

Efforts to Compare Methods

Comparison of size-fractionated chlorophyll estimated using HPLC with cell counts from flow cytometry and microscopy



By dividing the size-fractionated chlorophyll concentrations by the cell counts in each size fraction, able to estimate a range of intracellular chlorophyll concentrations for the three size classes.

Brotas et al. 2013

Recommendations

- No one method is entirely dependable – Use of any one method along would results in an incomplete or partially unambiguous characterization of the phytoplankton community
- Incorporating various methodologies leads to more complete diagnosis of the community
- Need for coordinated efforts to compile and generate comprehensive *in situ* datasets (not just for HPLC) for assessing composition
- Provide best practice guidance to merge the different types of datasets (HPLC, microscopy, flow cytometry) into an integrated product that encompasses different ways of grouping phytoplankton.

Recommendations

Methods:

- Methods to **convert from *in situ* data to phytoplankton biomass or fractions** should be assessed and protocols for merging different datasets (e.g., HPLC, microscopy, ...) should be formulated.
- **Differences in particle imaging and identification technologies** (e.g., holography, flow cytometry, flow cam, etc.) need to be assessed.
- **Coincident IOP** data with phytoplankton group measures need to be curated.
- Develop **standardized protocols** for all phytoplankton group relevant measurements.

Datasets:

- Specific **comprehensive datasets** should be compiled that include coincident IOPs, AOPs, and phytoplankton composition that serve as a resource for PFT algorithm development, refinement, and validation, and improve the ability to inter-compare validation metrics.
- **Standardization** of data products, quality, nomenclature, and format among different databases should be assured to enable easy compilation and expansion.

Bracher et al. 2017

Recommended Observations

Key observables to characterize phytoplankton communities:

- Phytoplankton pigments from HPLC, phycobilins from spectrofluorometry
- Phytoplankton cell counts and ID, volume/carbon estimation and imaging (e.g. from flow cytometry, FlowCam, FlowCytobot type technologies)
- Inherent optical properties
- Hyperspectral radiometry
- Particle size distribution
- Size-fractionated measurements of pigments and absorption
- Genetic/-omics data

Bracher et al. 2015

Questions

- With many different definitions of PFT, which metrics best serve which questions?
- Is there any site or effort that has simultaneously observed all of the recommended types of data?
- Few of these methods can be directly compared. So, how do we compare? How do we know which method is truth?
- How can we integrate observation types?
- Which set of observations are most comprehensive and how can we ensure integrated methods capture the continuum of the whole phytoplankton community assemblage?