How can Imaging Flow Cytometry serve Ocean Color Science?

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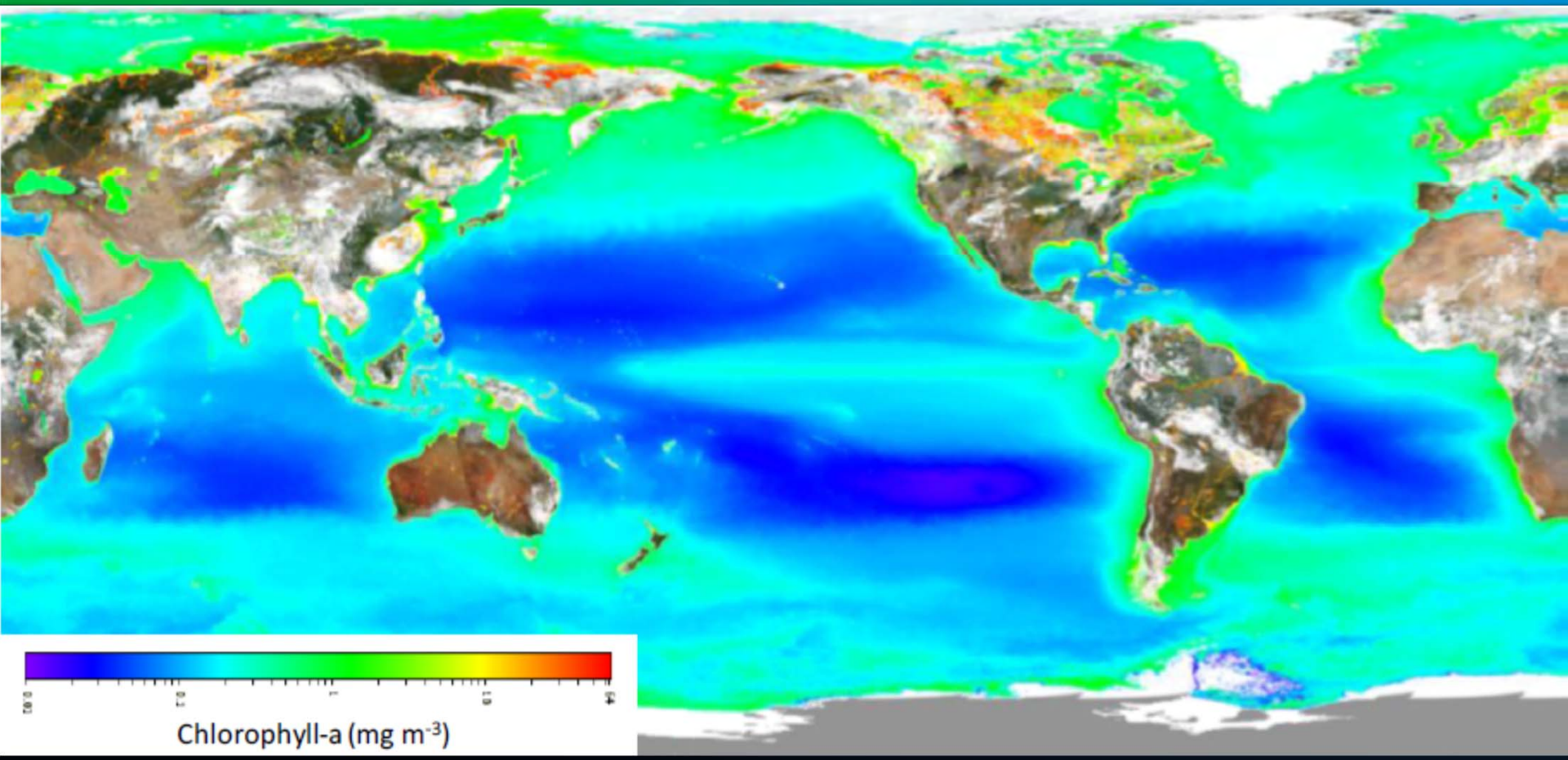
Remote Sensing of Ocean Color for Phytoplankton Observation

Global Upper Ocean Chl a



Global average Chl *a* distribution by VIIRS

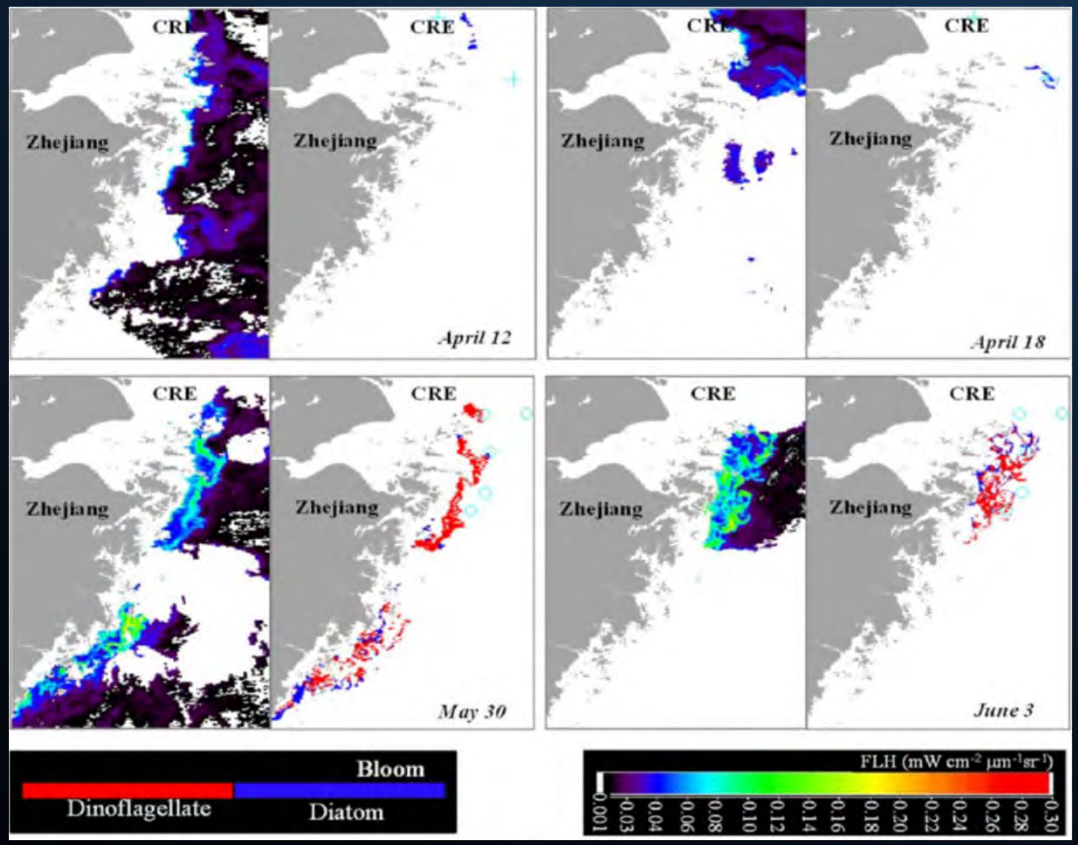
Image credit NOAA STAR Ocean Color

Detection of Phytoplankton Composition

Dominant Blooming Group

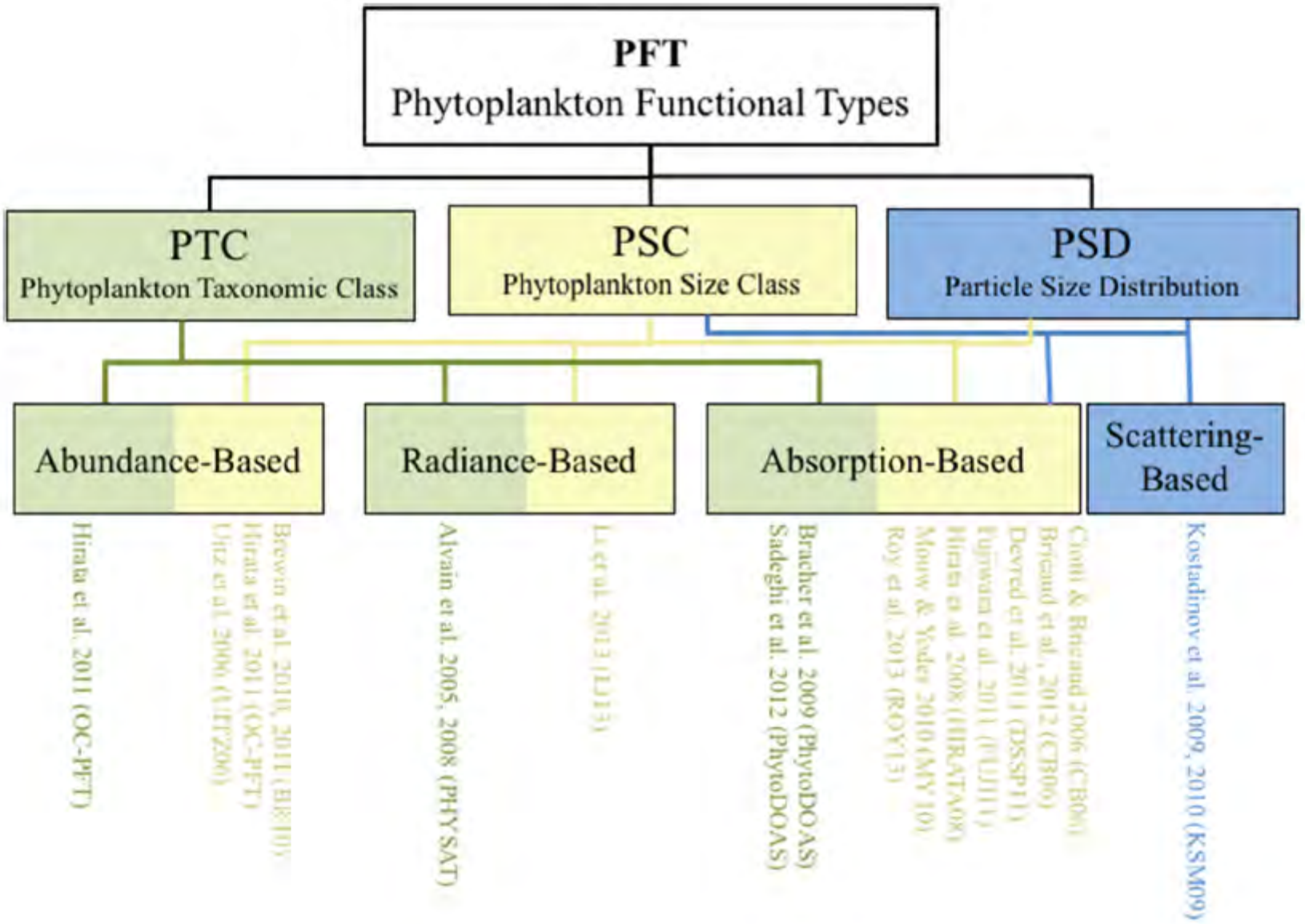
Bloom possibly caused by coccolithophorids, by MODIS-Aqua @ Bay of Biscay, France, 2004

Multiple Phytoplankton Functional Types



Shang, SL *et al.* J. Geophys. Res. 2014.

Phytoplankton Functional Type Algorithms



Mouw, C.B., et al., Frontiers in Marine Science, 2017.

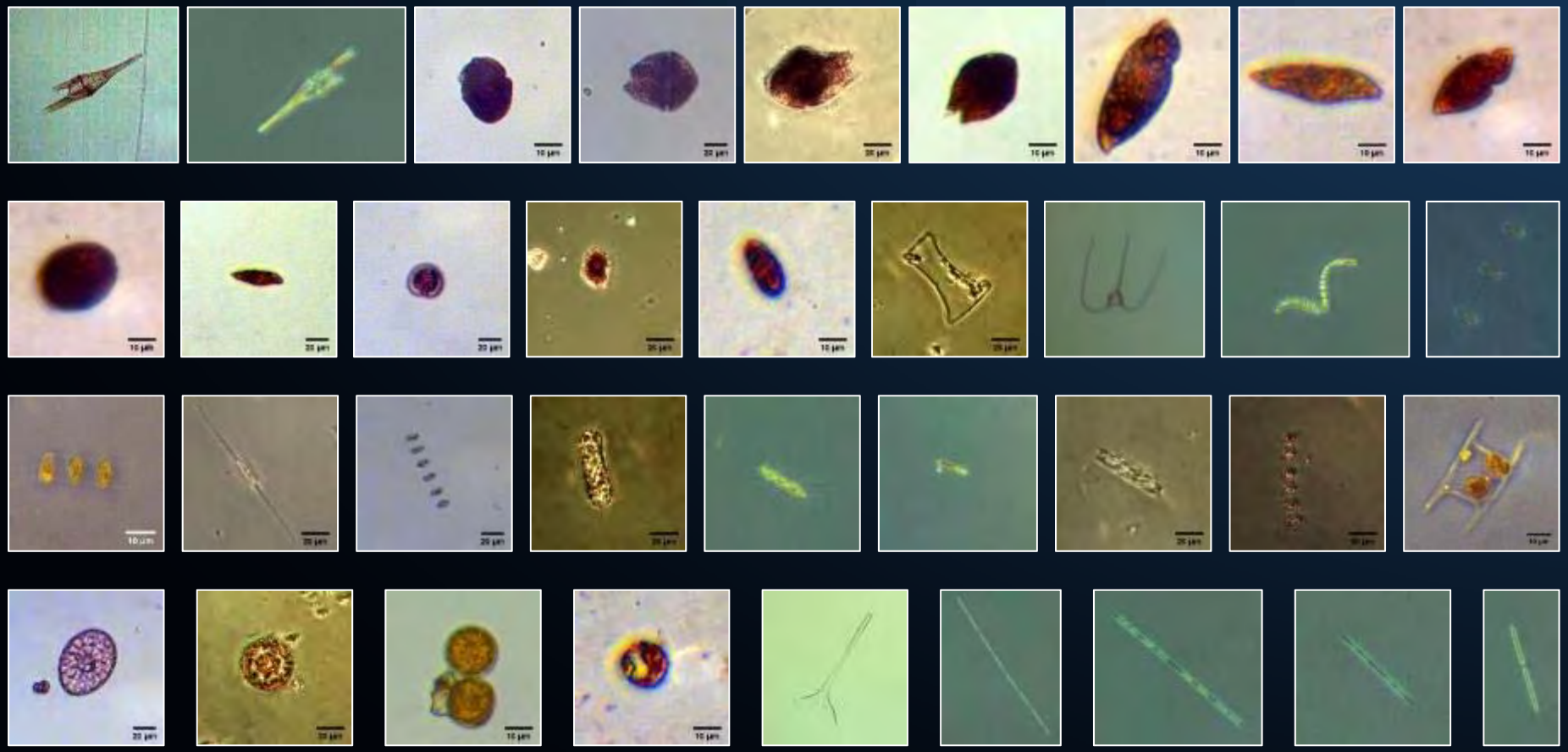
In situ Methods of Measuring Phytoplankton Functional Types: Providing the Sea-truth!

Table 2.3 Approaches for characterizing phytoplankton, with summaries of advantages, limitations, and relevance to advancing remote sensing applications.

Method	Advantages	Limitations	Relevance to Remote Sensing
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 - Identification of small cells (picoplankton) to species level difficult - Sensitive to methods used to collect and concentrate cells - 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
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 - 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
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 now available - Method development and testing time consuming - Relatively expensive and requires specialized equipment - Assumptions required to convert to biomass or size structure	- Largely unsensed
Inherent Optical Properties	- 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
Fluorescence Excitation and Emission 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
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

Chapter 2, P37

Phytoplankton

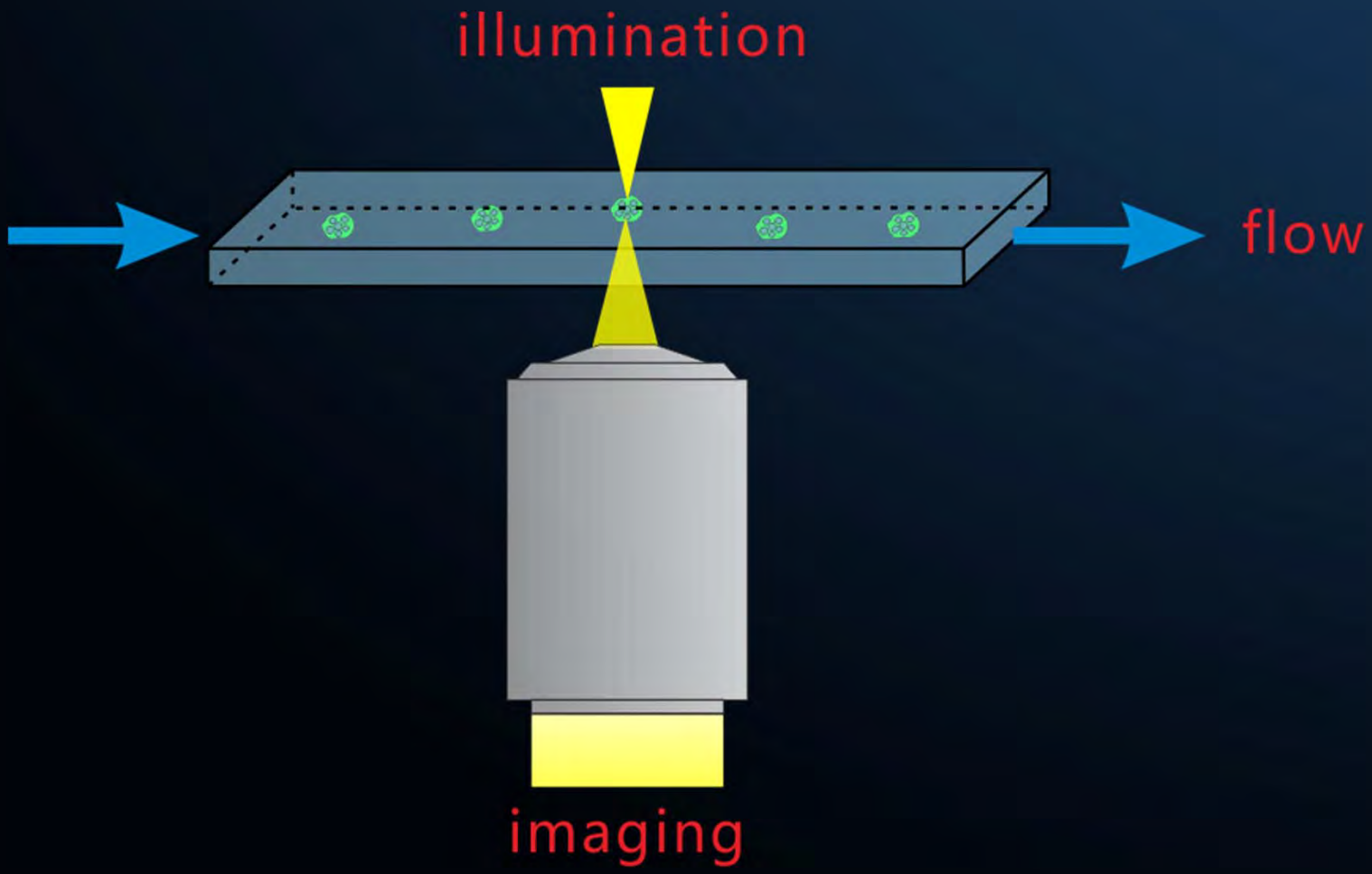


Great Heterogeneity and Diversity in
Size, Morphology, Mobility, Abundance, Distribution, Pigment composition, Physiology
Non-phytoplankton……

In situ Methods for Phytoplankton Observation

	Methodologies	Field-deploy Capability	Field-deploy Time Span	Quantitation Capability	Taxonomy Specificity	Sizing Capability	Detection Throughput	Sampling Frequency
Water Ensemble	Fluorimetry	★★★★★	★★★★★	★	★	×	★★★	★★★★★
	Spectrophotometry	★★★★	★★★★	★	★	×	★★★	★★★★★
	HPLC	★	NA	★	★	★	★★★	★
	Molecular Methods	★	★	★	★★★★★	×	★★	★
Cellular Level	Manual Microscopy	★★	★	★★★	★★★★	>10μm	★	★
	Automated Microscopy	★	NA	★★	★★★	>10μm	★★	★★
	Flow Cytometry	★★★	★★★	★★★	★	0.5μm-10μm	★★	★★★
	Imaging Flow Cytometry	★★★	★★★	★★★	★★★	>10μm	★★★	★★★

Imaging-in-flow Concept

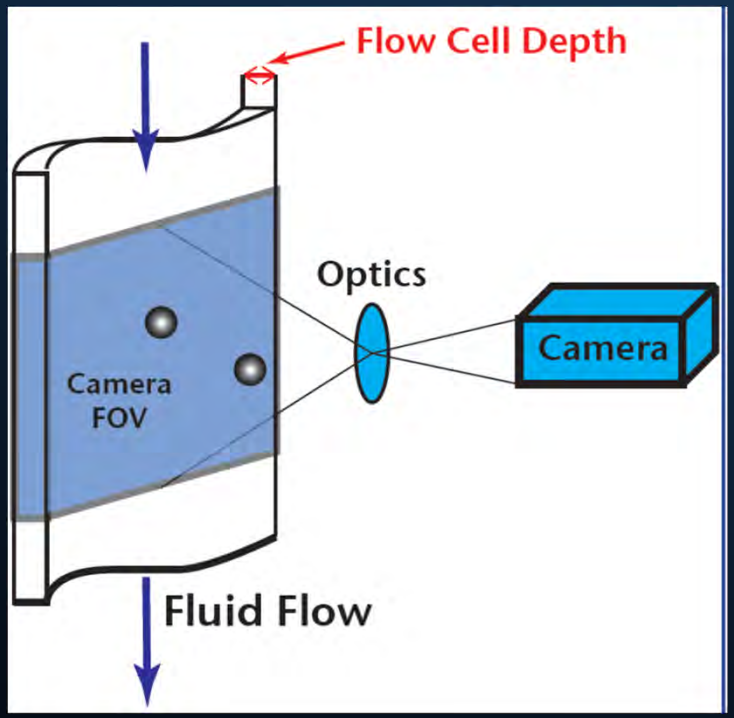


Combining Optical Microscopy with Flow Cytometry

Bright-field IFC: FlowCAM


Figure 1: Typical FlowCAM algae images acquired from an ocean sample

Image credit: Fluid Imaging Technologies



Christian K. Sieracki, et al., Marine Ecology Progress Series, 1998.

Challenges for High-throughput Phytoplankton BF-Imaging

Imaging speed

vs.

Imaging quality

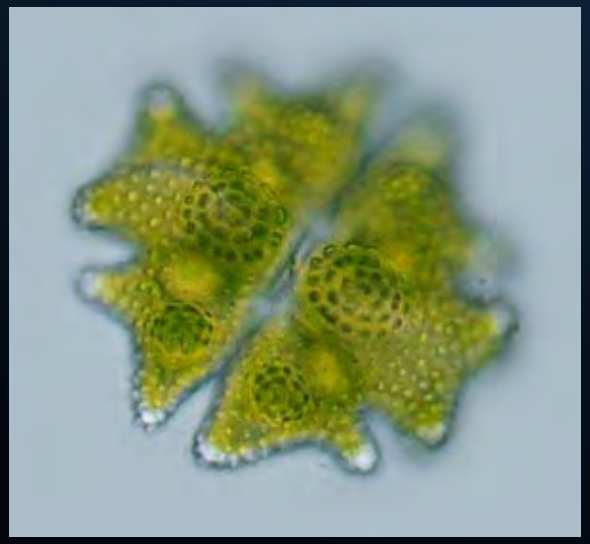
– Lateral-motion blur

Higher Mag.(resolution)

vs.

Shallower DOF

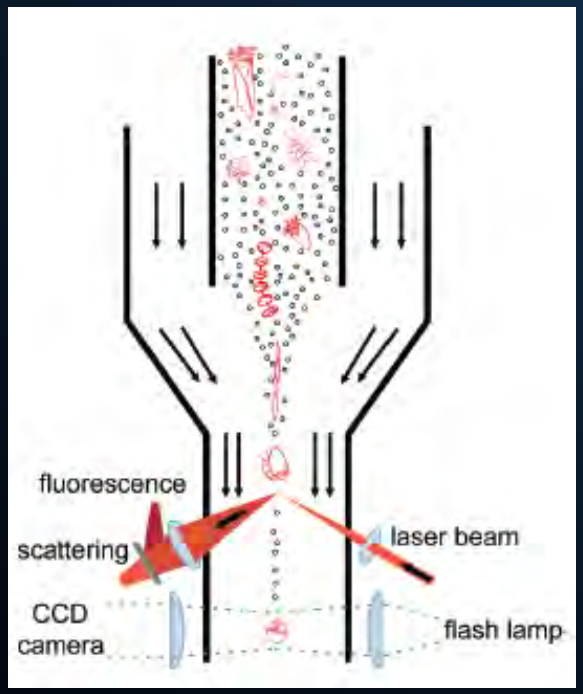
– Axial defocus blur

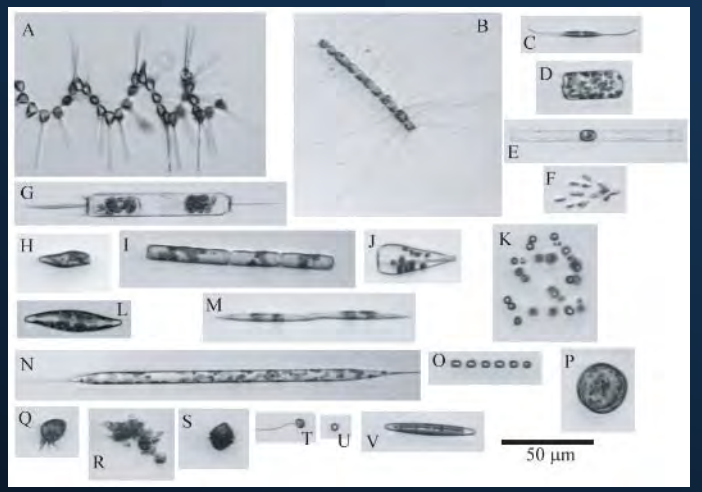


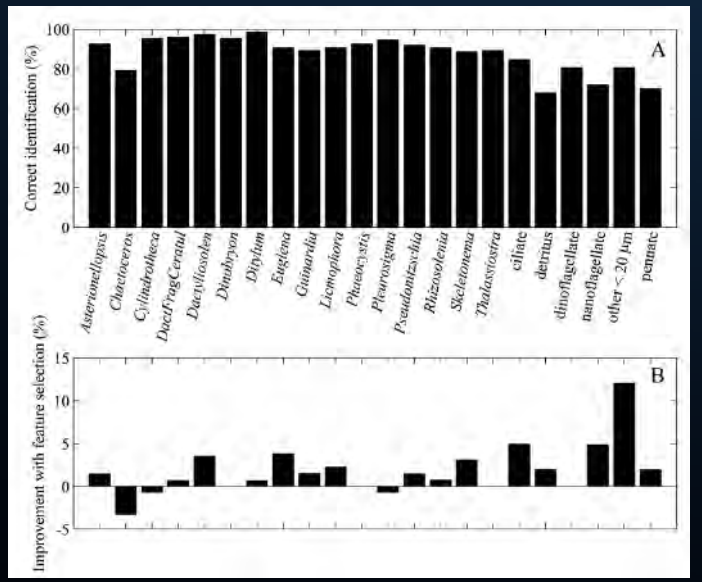
Images from internet for conceptual illustration

BF-IFC: Imaging FlowCytobot (IFCB)

Image credit: McLANE







1. Olson R J, Sosik H M. Limnology and Oceanography: Methods, 2007.
2. Sosik H M, Olson R J. Limnology and Oceanography: Methods, 2007.

Characteristic of Bright Field-IFC

Pros:

- Morphology analysis consistent with Taxonomist experience
- LIF of chl *a* trigger
- FC function integrated (IFCB)
- Field/in situ deployment
- Specialized image analysis algorithms

Cons:

- Limited throughput
- Low sensitivity
- Limited sizing range
- Complex fluidic manipulation
- Particle enumeration calibration needed

Amnis Series MS-TDI-IFC

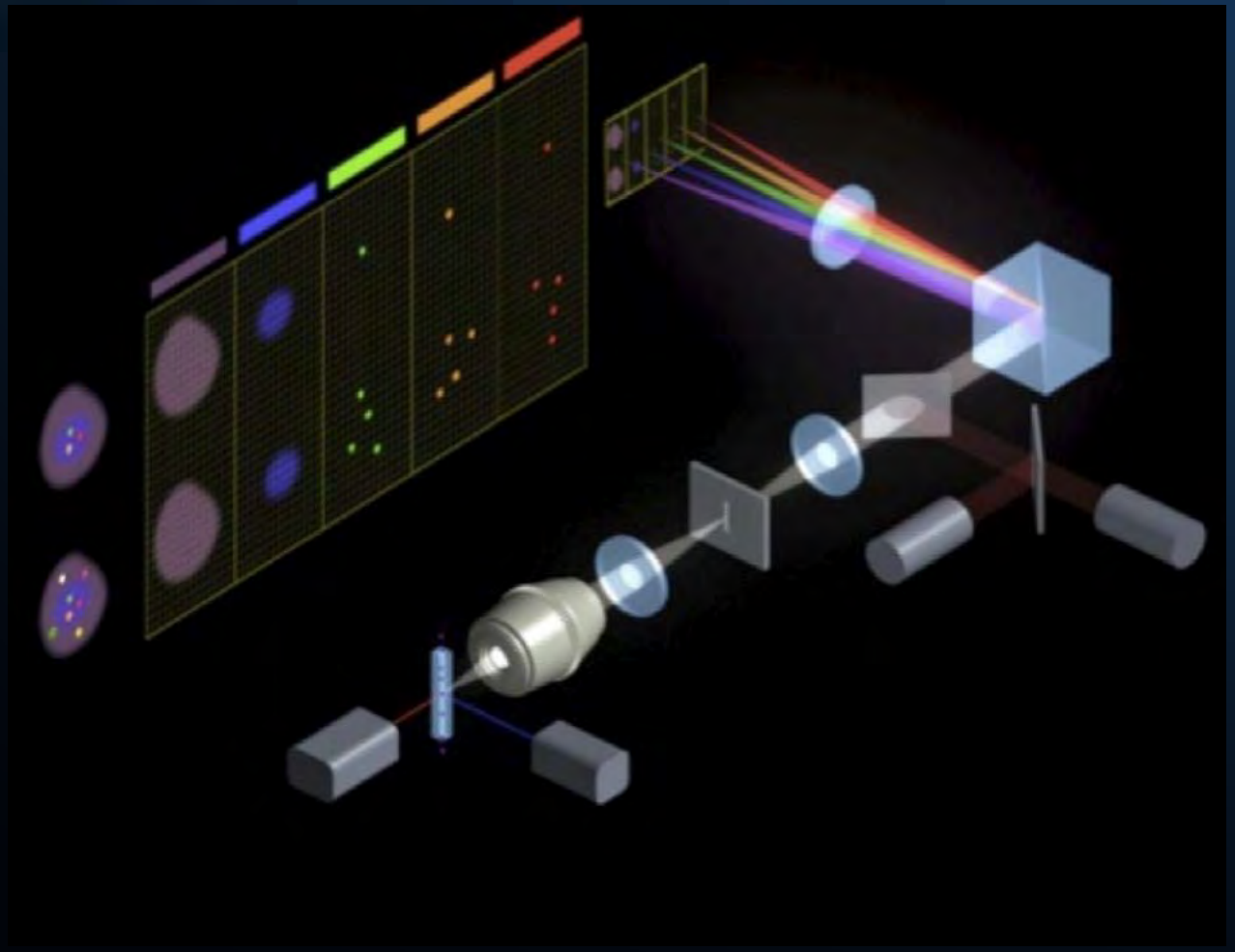


Image credit: Luminex Corporation

Amnis Series MS-TDI-IFC

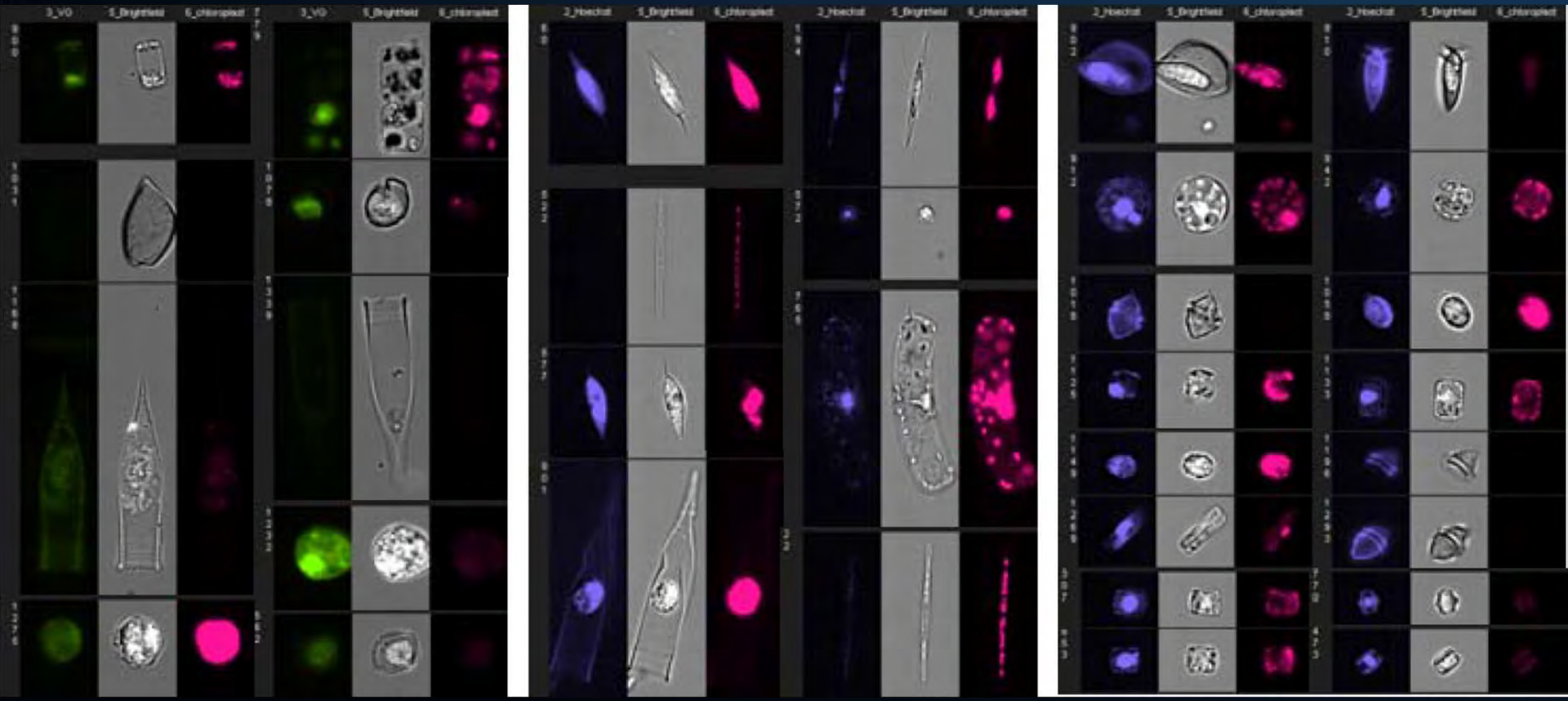


Image credit: Luminex Corporation

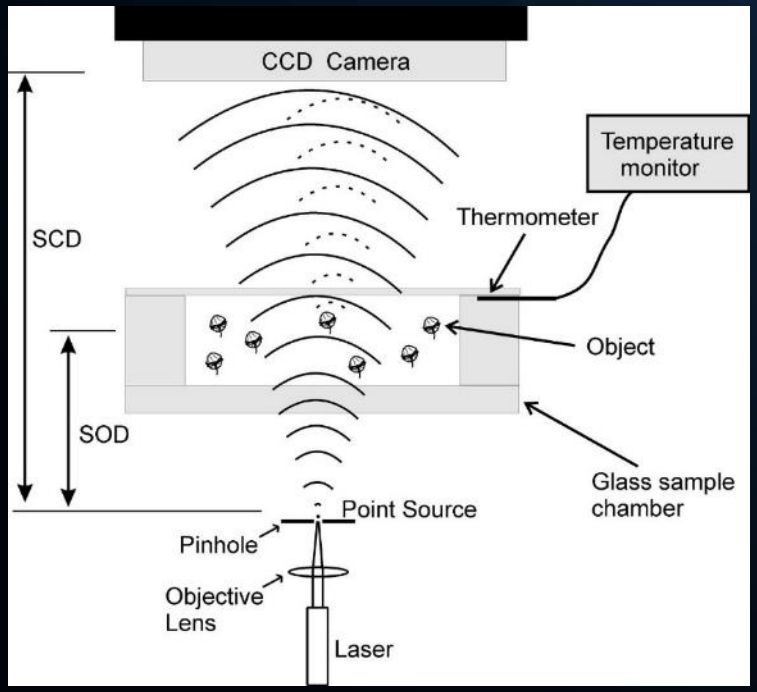
Characteristic of MS-TDI-IFC

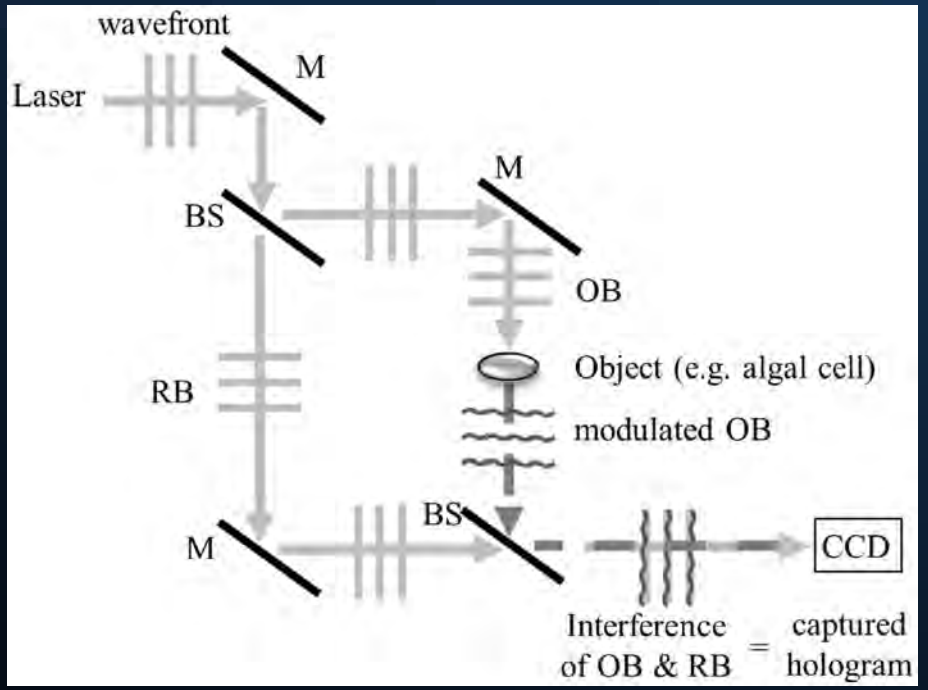
Pros:

- Multispectral multi-modal (fluorescence) imaging
- Time delay integration (TDI) imaging
- Extended DOF by wavefront coded element and computation

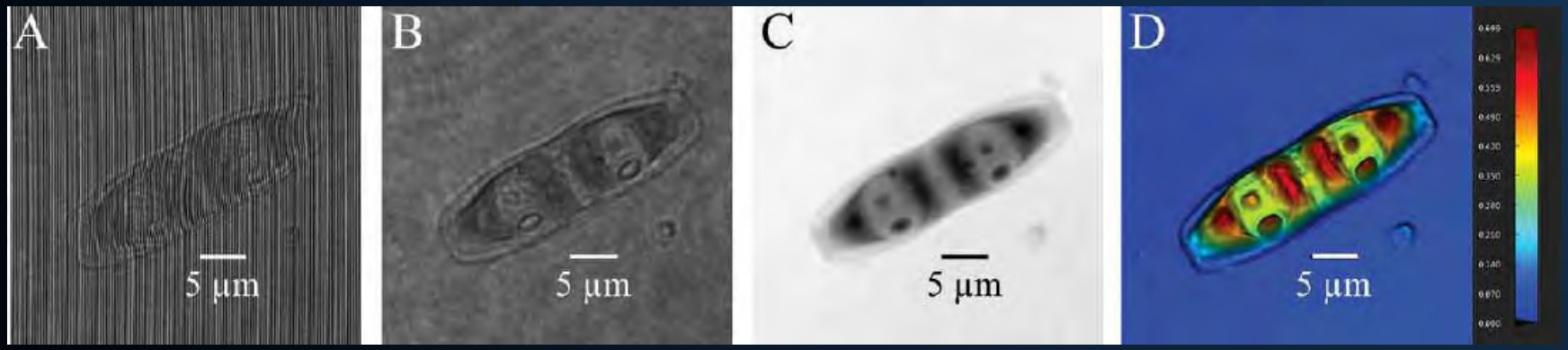
Cons:

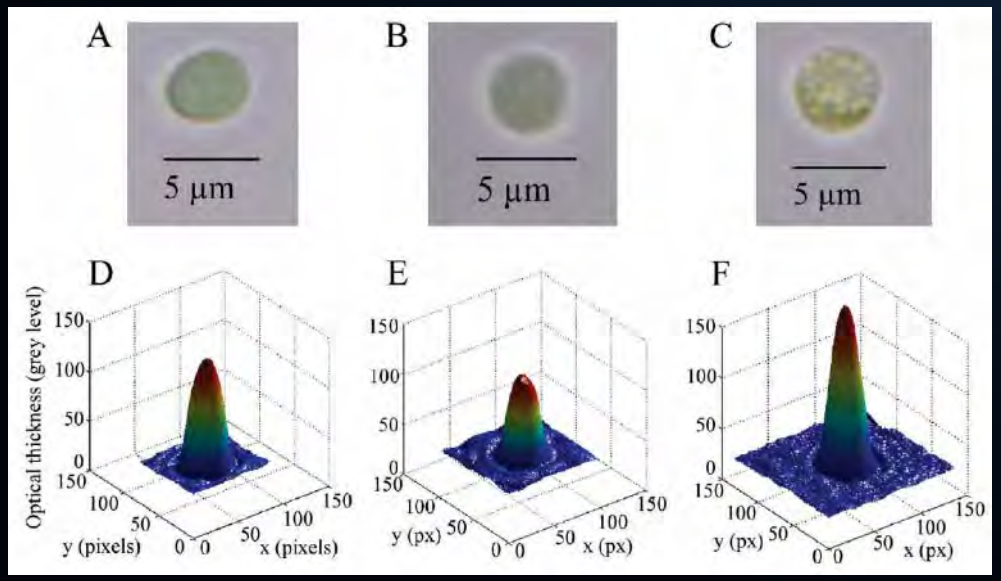
- Limited throughput
- Narrow sizing range
- Hydrodynamic focusing needed
- Complexity/High cost
- Not for field/in situ
- Not specialized for natural phytoplankton water sample

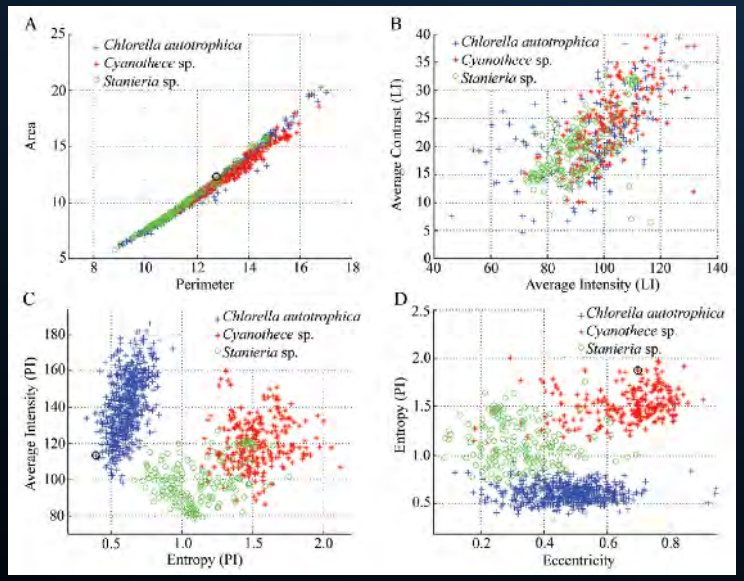

CCD Camera
Temperature monitor
Thermometer
SCD
Object
SOD
Glass sample chamber
Point Source
Pinhole
Objective Lens
Laser


wavefront
Laser
M
BS
M
OB
Object (e.g. algal cell)
RB
modulated OB
BS
M
CCD
Interference of OB & RB
=
captured hologram

DH-IFC

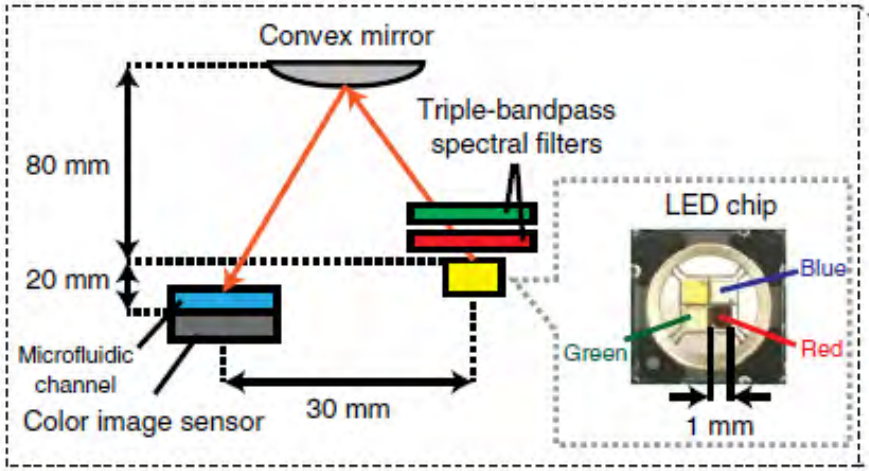


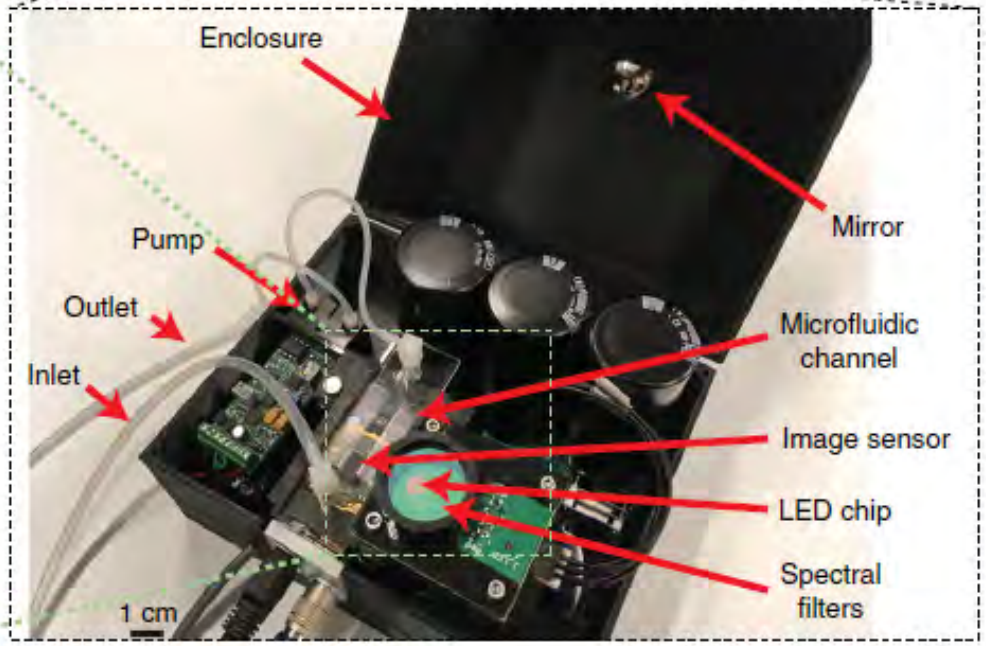




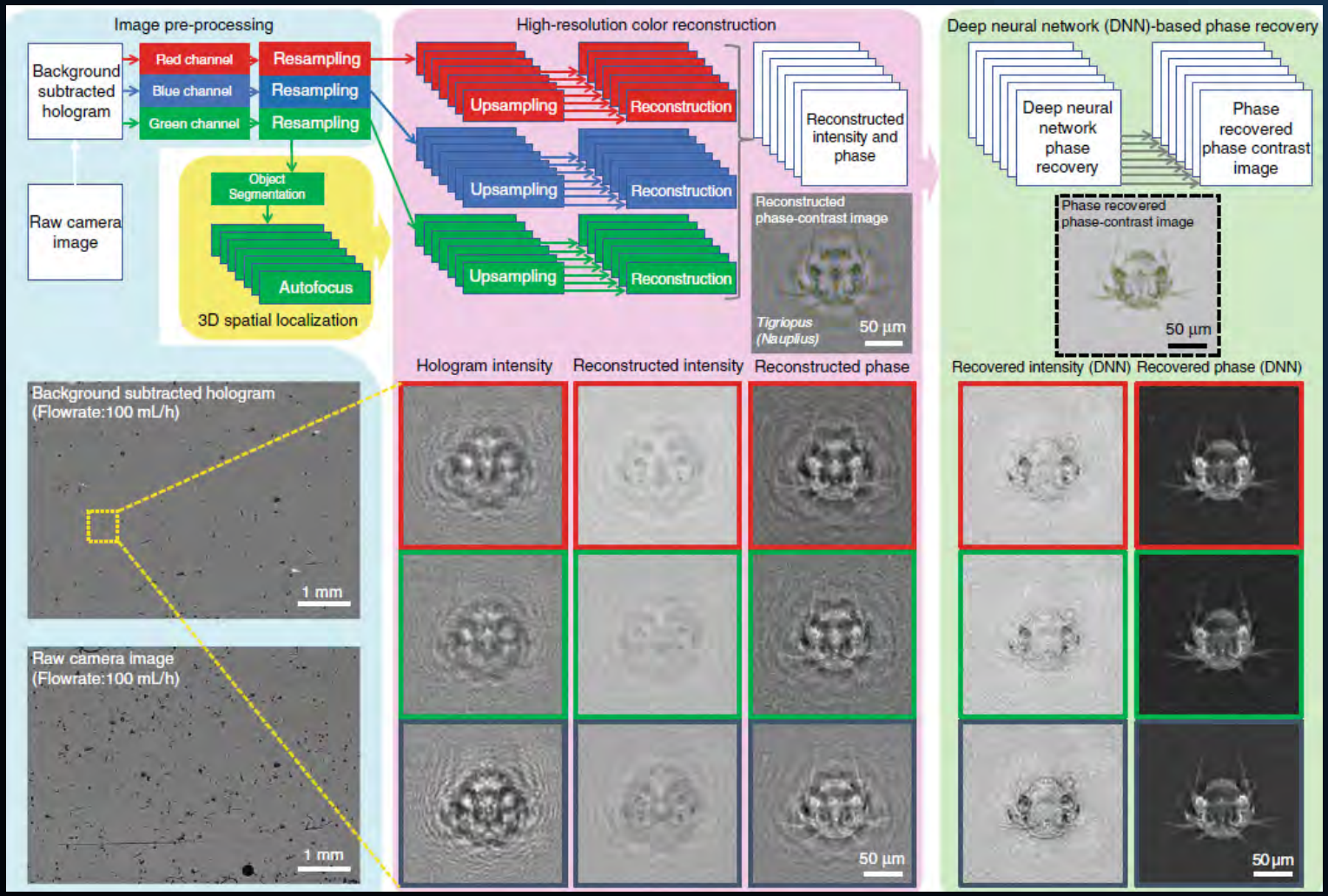
Zetsche Eva-Maria, et al., Limnology and Oceanography: Methods, 2014

AI-enabled DH-IFC





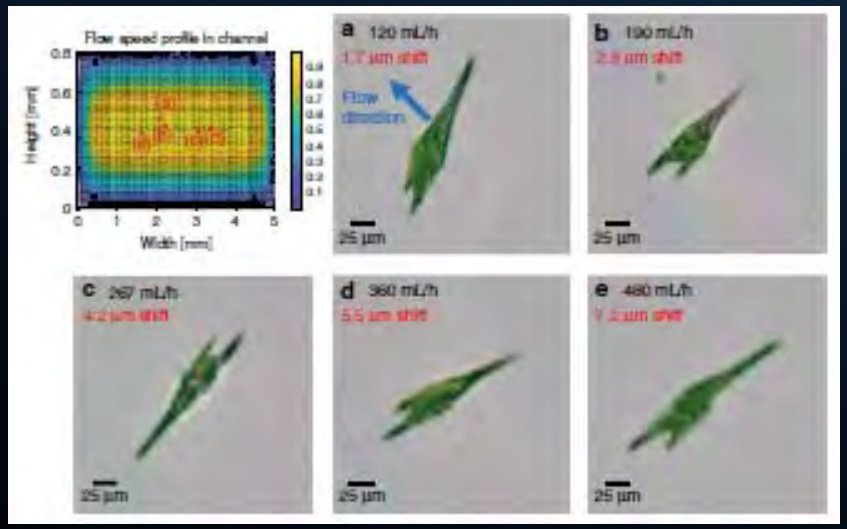
AI-enabled DH-IFC



Grcs, Z., et al., Light Sci Appl, 2018

AI-enabled DH-IFC

Phase recovered phase-contrast images@1.6mL/min



Motion-blur emerges with increase in throughput

Grcs, Z., et al., Light Sci Appl, 2018

Characteristic of DH-IFC

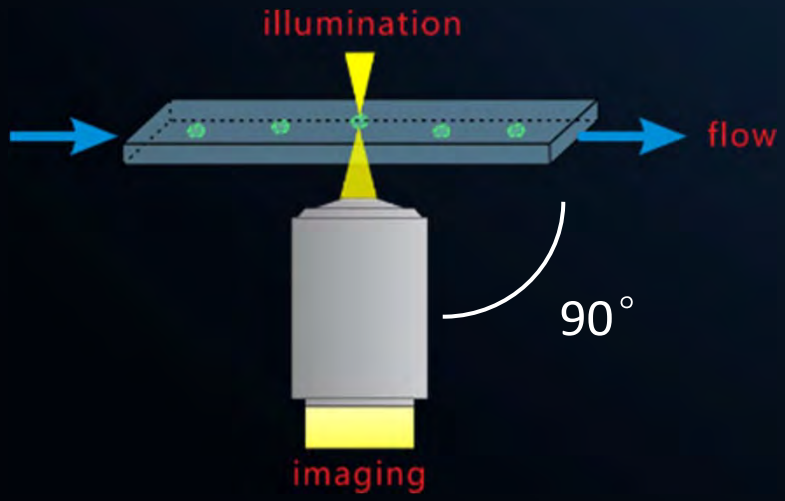
Pros:

- New features of phase beyond morphology
- Wider sizing range by extended DOF (computational refocusing)
- Simpler optics for field/in situ instrumentation
- Computational demand released by DNN and GPU

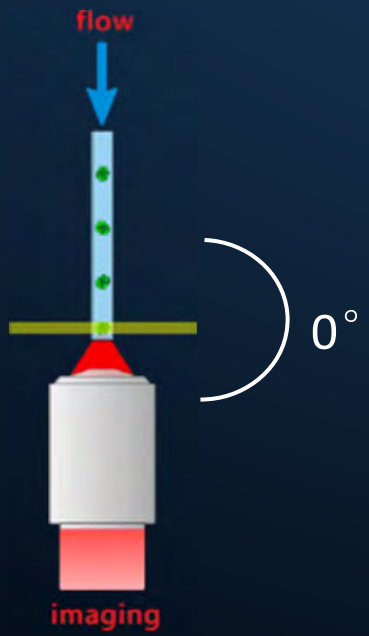
Cons:

- Throughput still limited by lateral motion blur
- Poor sensitivity for pico-
- Poor phyto-specificity
- Particle enumeration calibration needed

Imaging-in-Flow: Concept Revisit

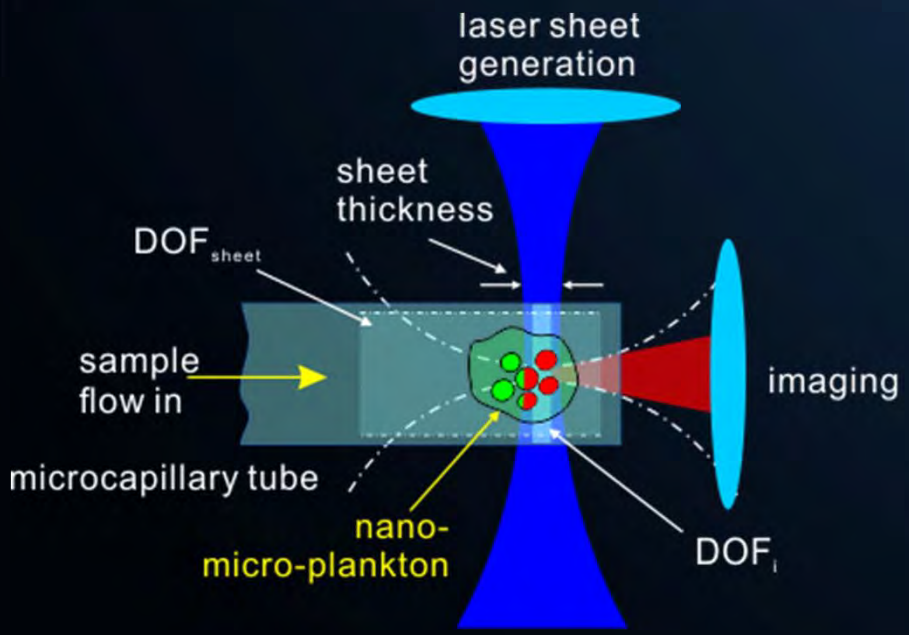


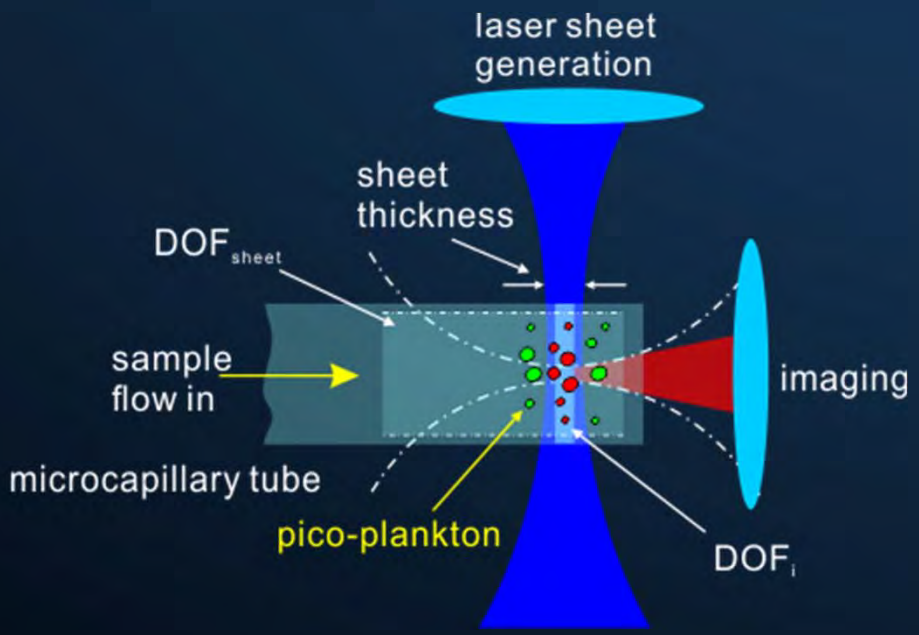
Flow through FOV laterally in/along focal plane

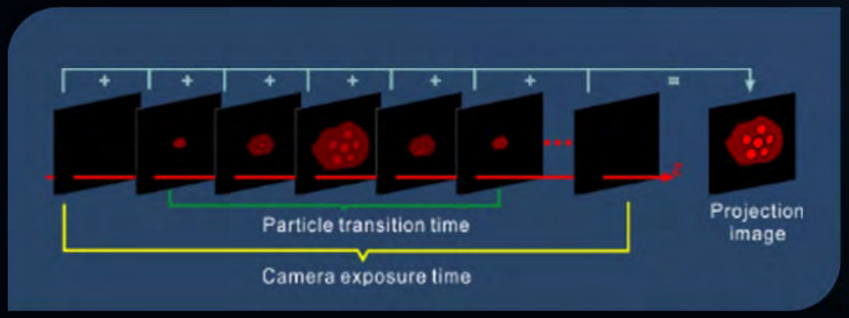


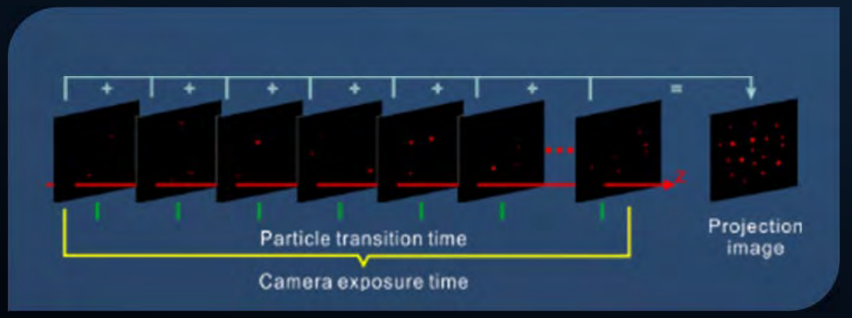
Flow through focal plane axially along imaging axis

Light-sheet Fluorescence IFC



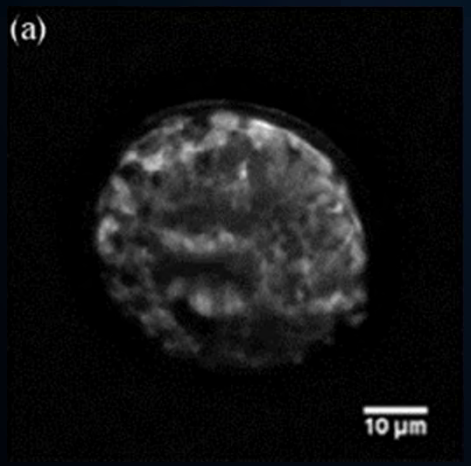


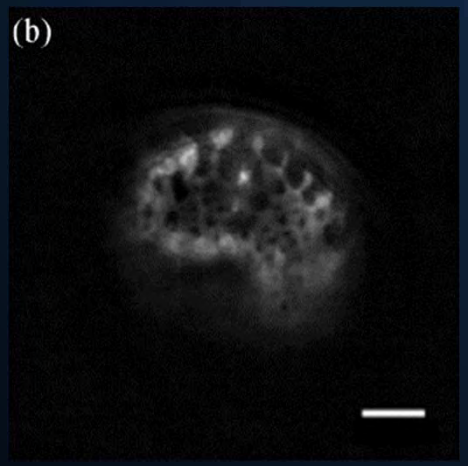


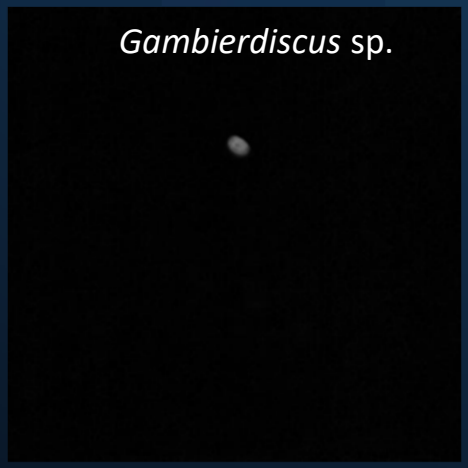


J. Wu, J. Li, and R. K. Y. Chan, Opt. Express, 2013.

3D Tomographic LSF-IFC





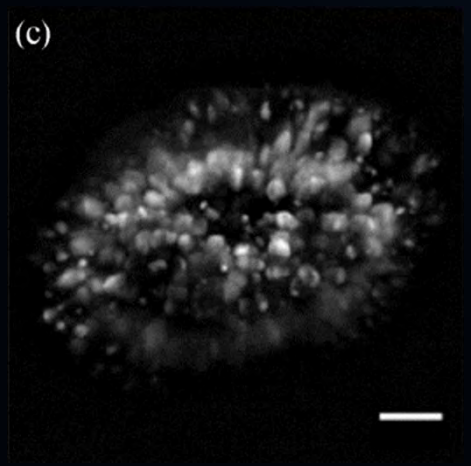


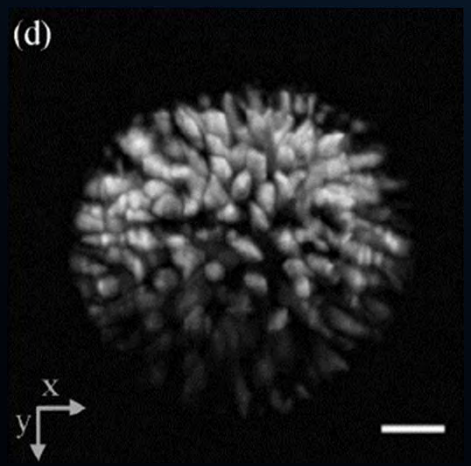
Mag: 40x

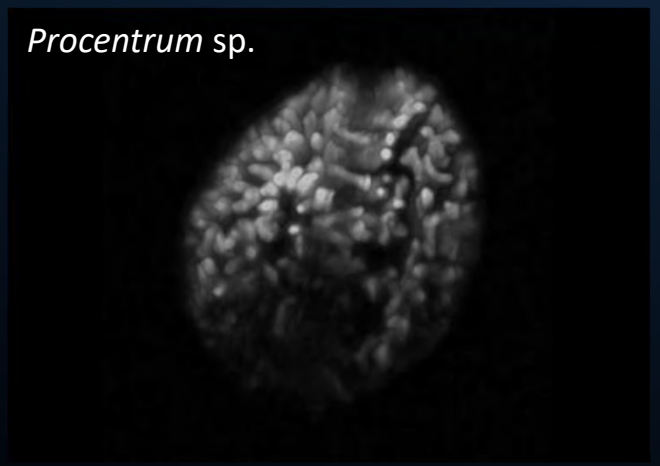
FOV:
33µmx33µm

Resolution:
Hz-0.75µm
Ax-2µm

Flow rate:
0.5µL/min

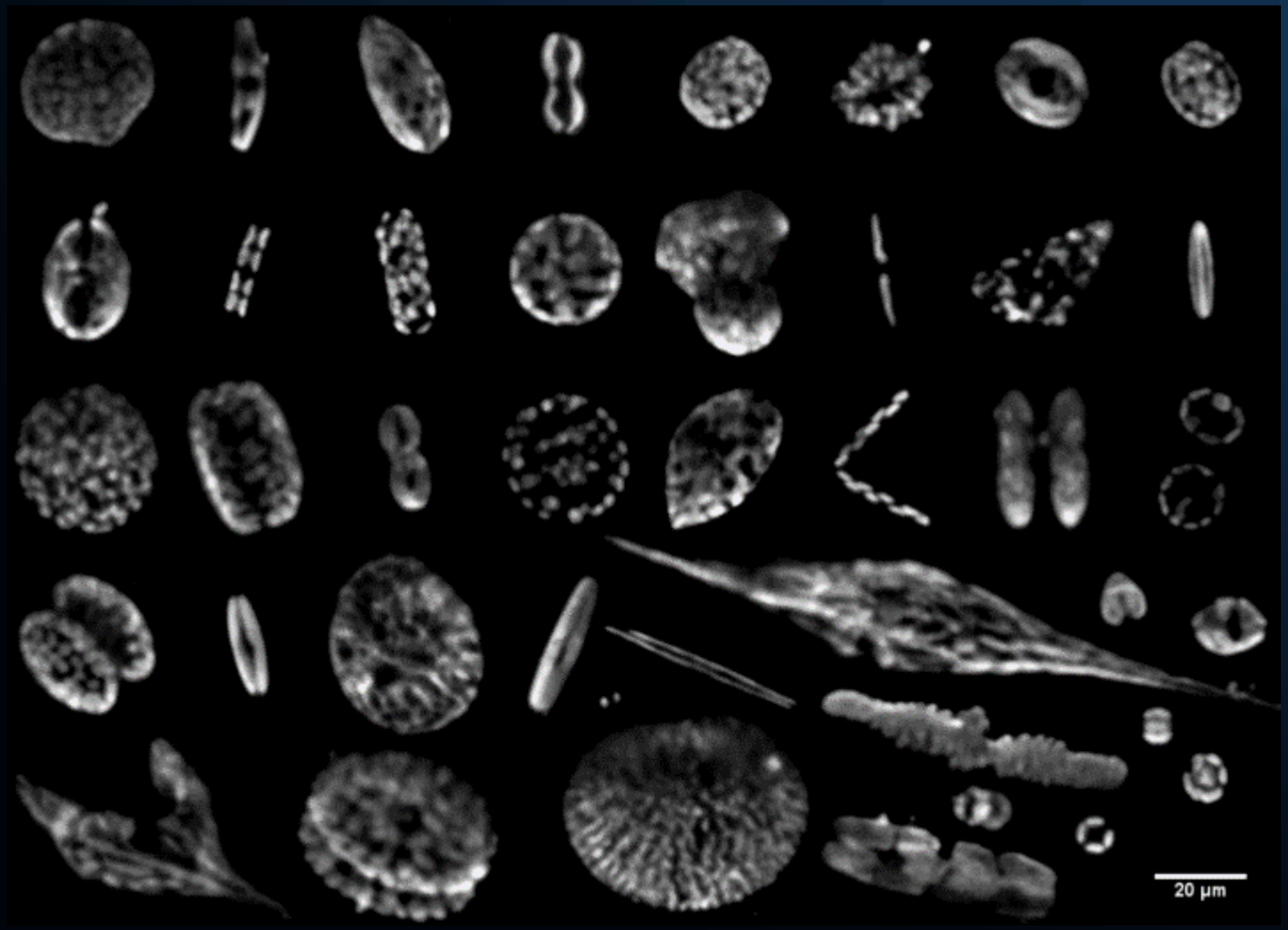






J. Wu, J. Li, and R. K. Y. Chan, Opt. Express, 2013.

2D Focus-Stacking LSF-IFC



Mag: 40x

FOV:
200μmx200μm

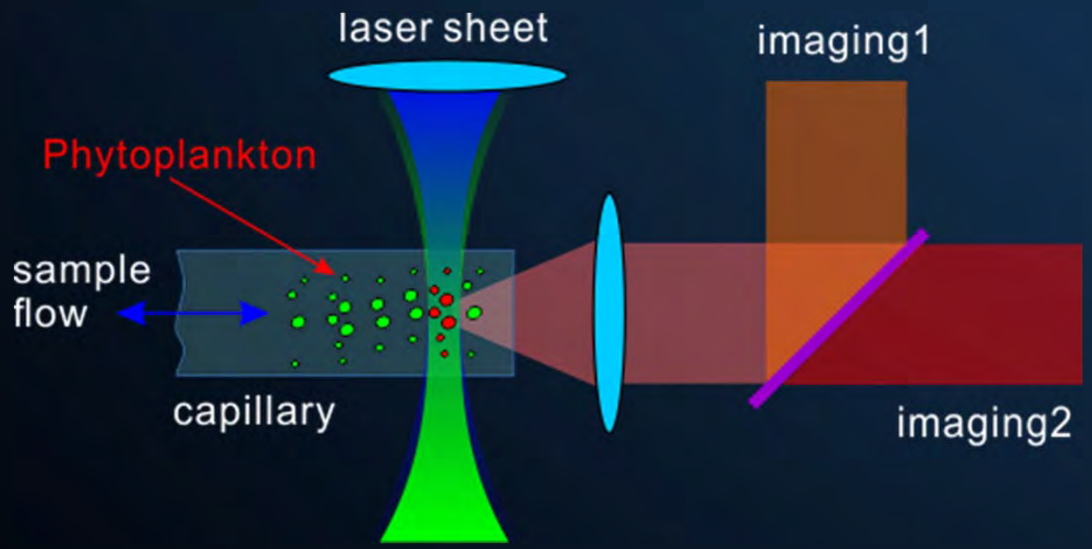
Resolution:
Hz-0.75μm

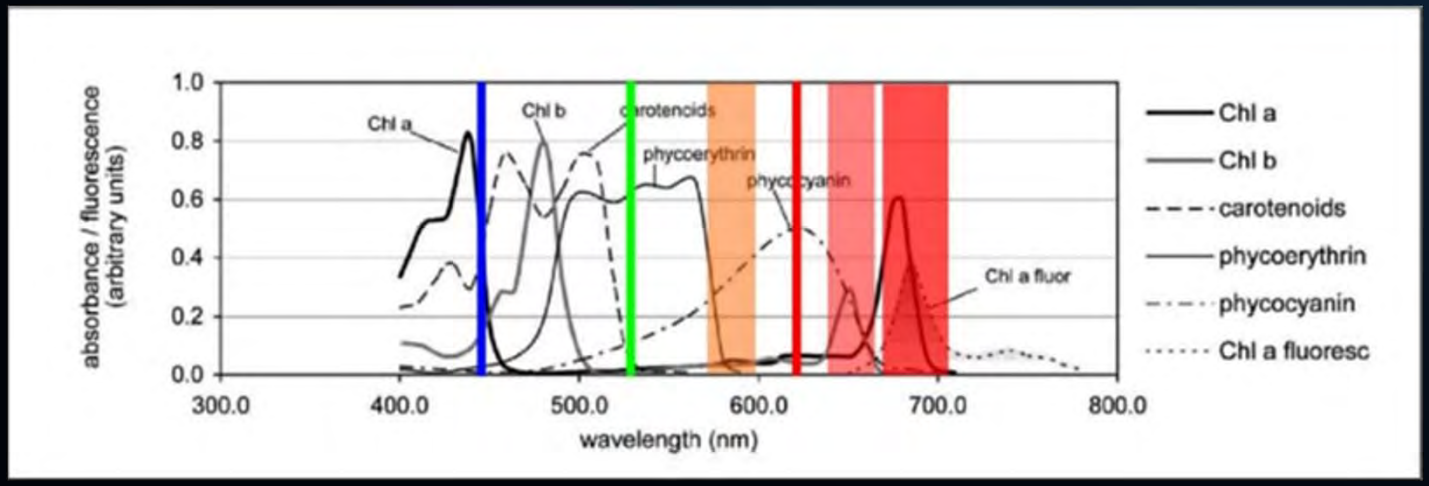
Flow rate:
1mL/min

2000times↑

Wu J.L, Chan R.K.Y., Optics Express, 2013.

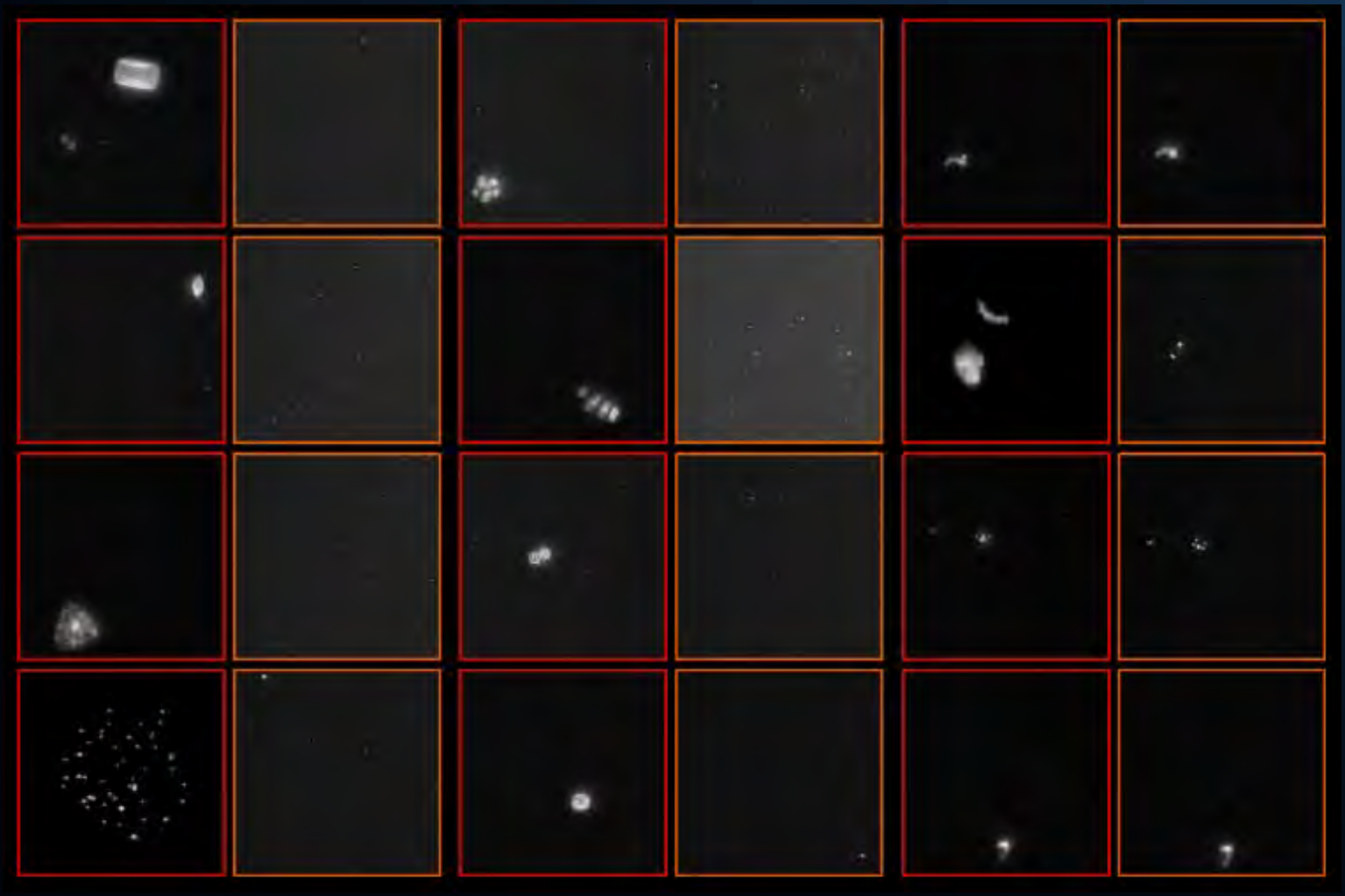
Two-color 2D LSF-IFC





Li JP, Xu ZN, Chan R, *Focus on Microscopy, 2016.*

Chl a-PE Imaging



Mag:20x

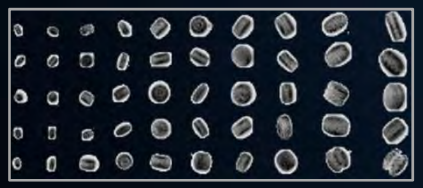
FOV:
300μmx300μm

Resolution:
Hz-1μm

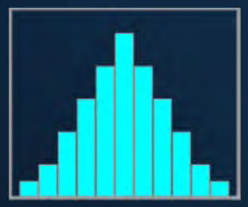
Flow rate:
1mL/min

Li JP, Xu ZN, Chan R, *Focus on Microscopy, 2016.*

Quantifying Phytoplankton Variables from Images



Identification



Sizing

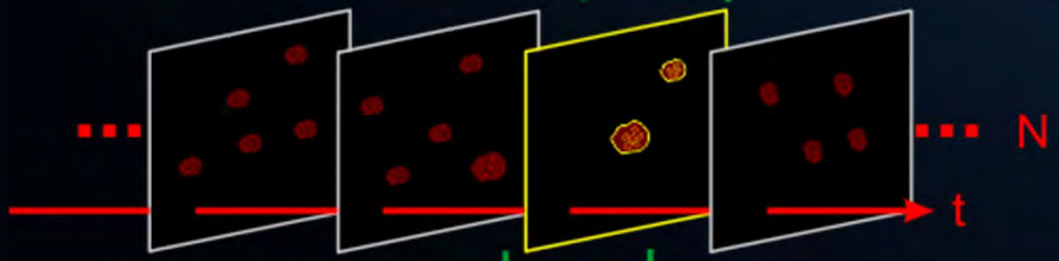
Chl *a* concentration

Size

Fluorescence

Particle#

d, I_f, and n_f



$V_f = Q \times t_{exp}$

Volume flow rate

Exposure time

Total particle #

Total fluorescence intensity

$$\frac{n_{total} = \sum n_f \,/\, I_{total} = \sum I_f}{V_{total} = \sum V_f = N \times V_f}$$

Total volume

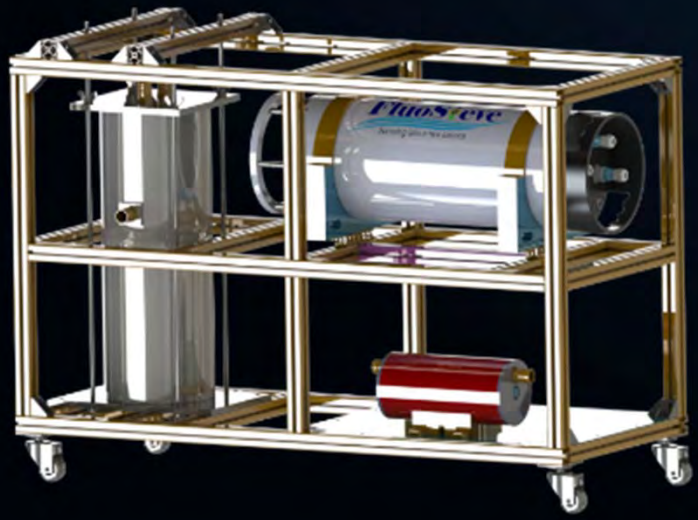
Abundance

LSF-IFC Features

- High imaging quality
  - High resolution/contrast
  - No motion-/defocusing-blur
  - 3D/2D imaging
- High throughput
  - Volume rate reaches to several ml/min for larger cells
  - Cell detection rate reaches to >10^5 counts/s for pico-
- Broad sizing range
  - ~1-300µm
  - Preservation of fragile colonial and chain species
- High counting accuracy
  - No cell enumeration calibration needed
- High sensitivity
  - Laser-induced fluorescence
  - Picophytoplankton detectable
- High specificity
  - Chl a autofluorescence imaging
  - Immune to bubbles and most non-phytoplankton particles
- Multi-color imaging
  - *Chla* channel for larger cell recognition
  - Pigment analysis for more
- Add-on function
  - Chl a fluorimetry with little CDOM contamination
- Methodological simplicity and flexibility for instrumentation!!!

Towards Field Deployment

2018: FluoSieve instrument



2019: Integrated system for ship-borne

Coastal Observatory

Research Vessel

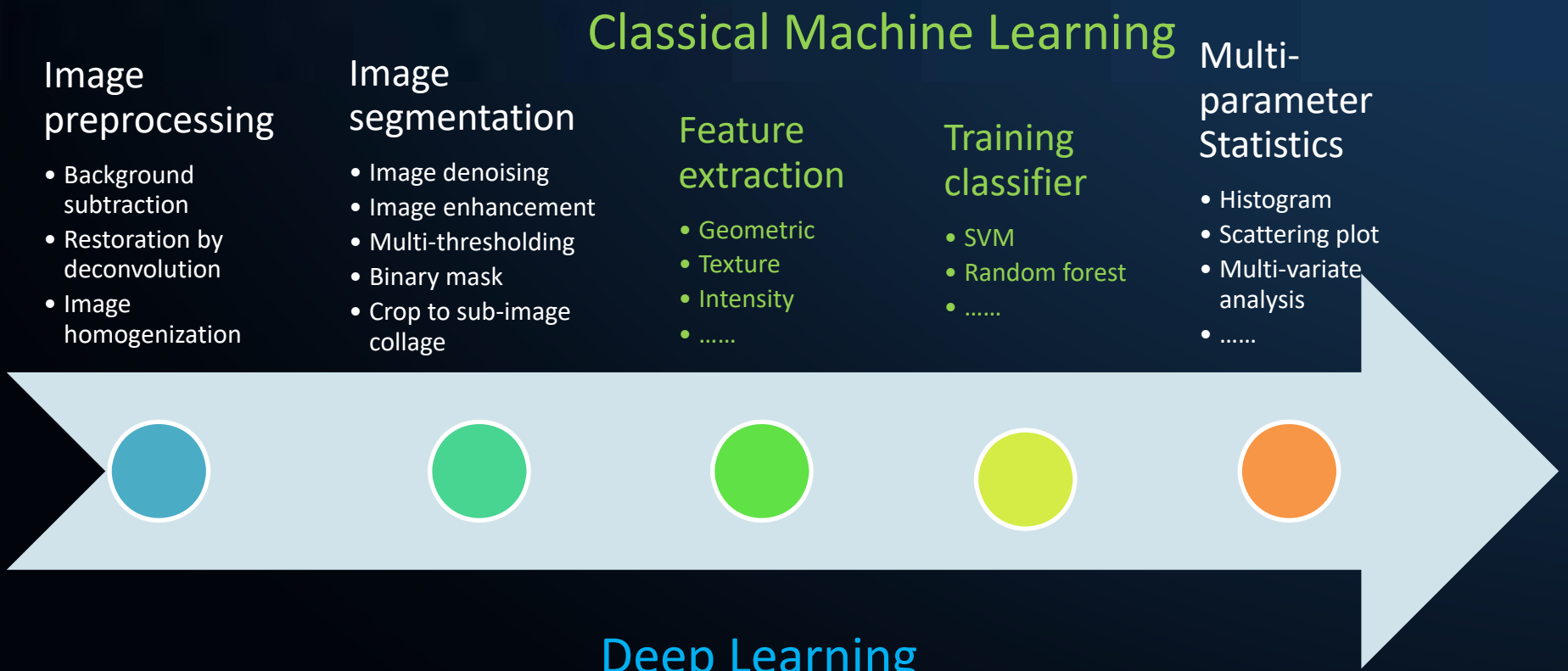
On-board Labs

Power & Seawater Supply

Computation & Communication

Ecological Buoy

Automatic Image Analysis

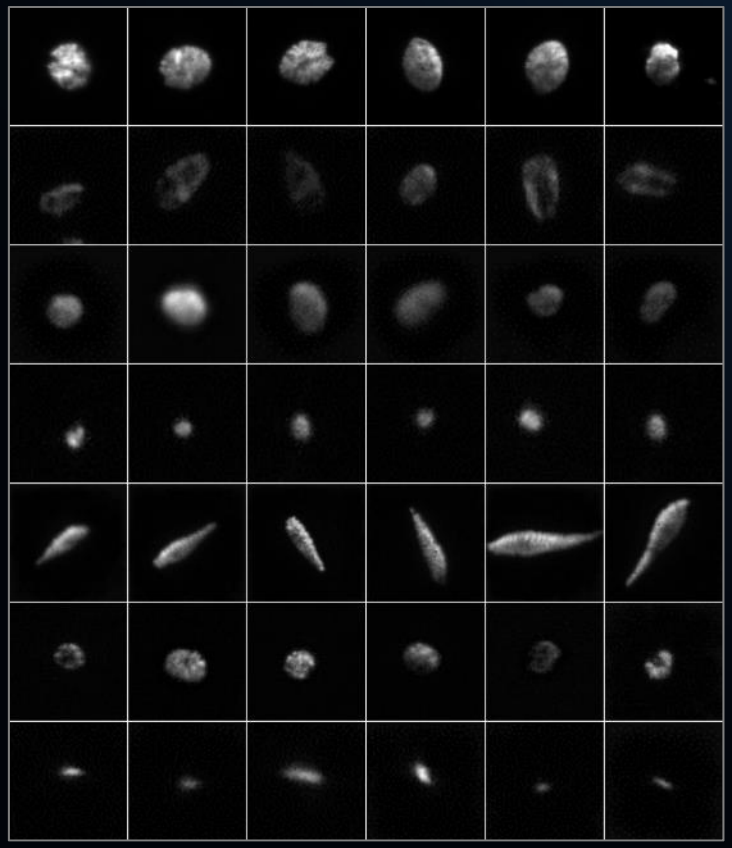


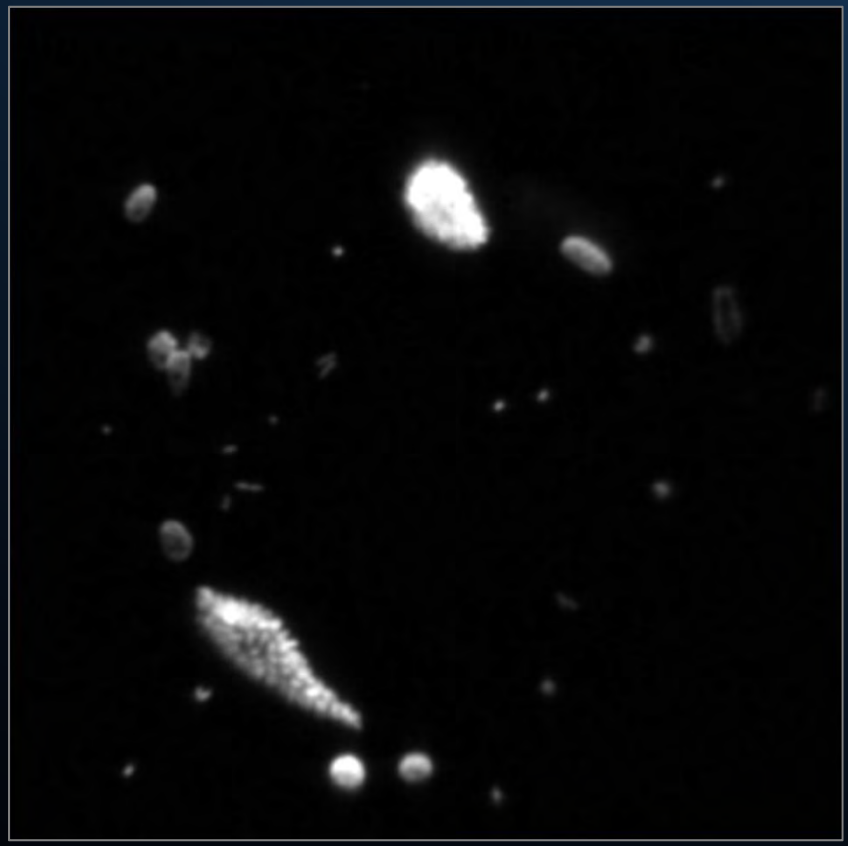
Deep Learning

- Large-scale database
- CNN modeling and training
- ……

Analysis Example

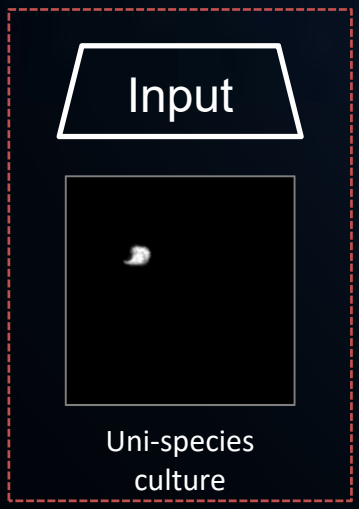
7 Cultured Species





Unpublished!

Analysis Example



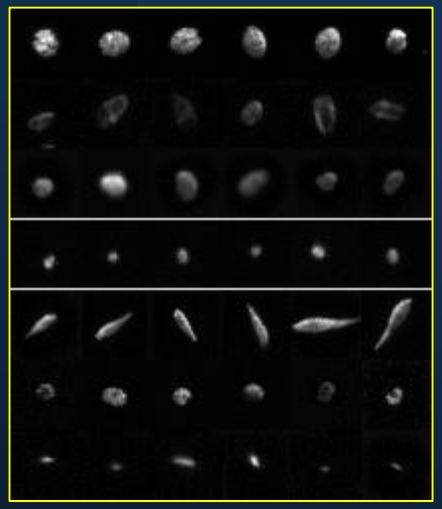


Image Database

Curvature
Local Binary Pattern

Class	1	2	3	4	5	6	7
							
SVM training	300	300	300	300	300	300	300
Testing	80	80	80	80	80	80	80
Accuracy	73%	78%	84%	81%	71%	76%	78%

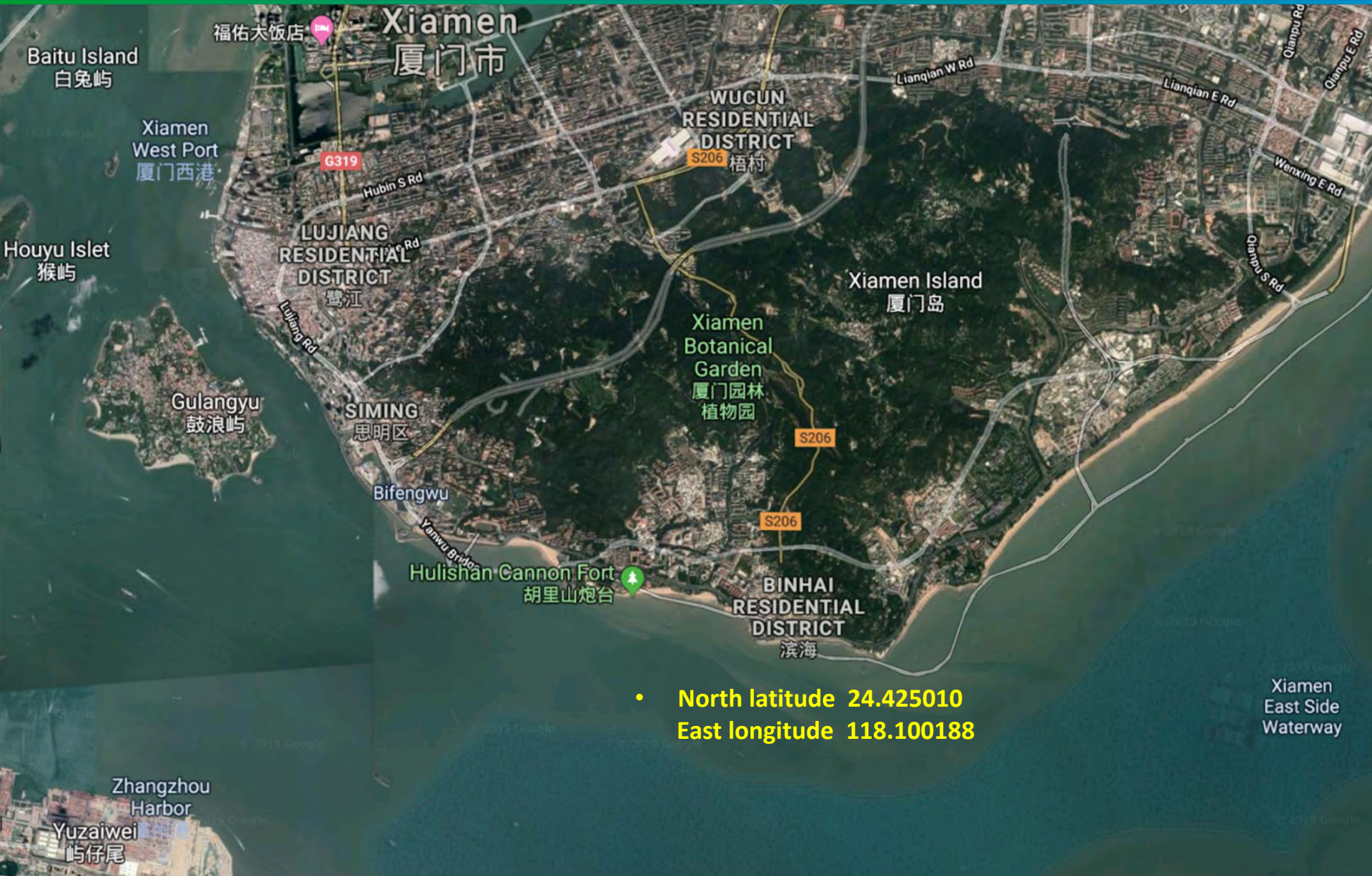
SVM Classifier

Cell	Area	ESD	La	Sa	R	P	In
	32192	202.45	375.26	113.68	3.30	1059	4092
	36096	214.38	375.37	129.22	2.90	1127	3114
	21760	166.45	214.78	135.68	1.58	706	3619

Geometric features for measurement

Unpublished!

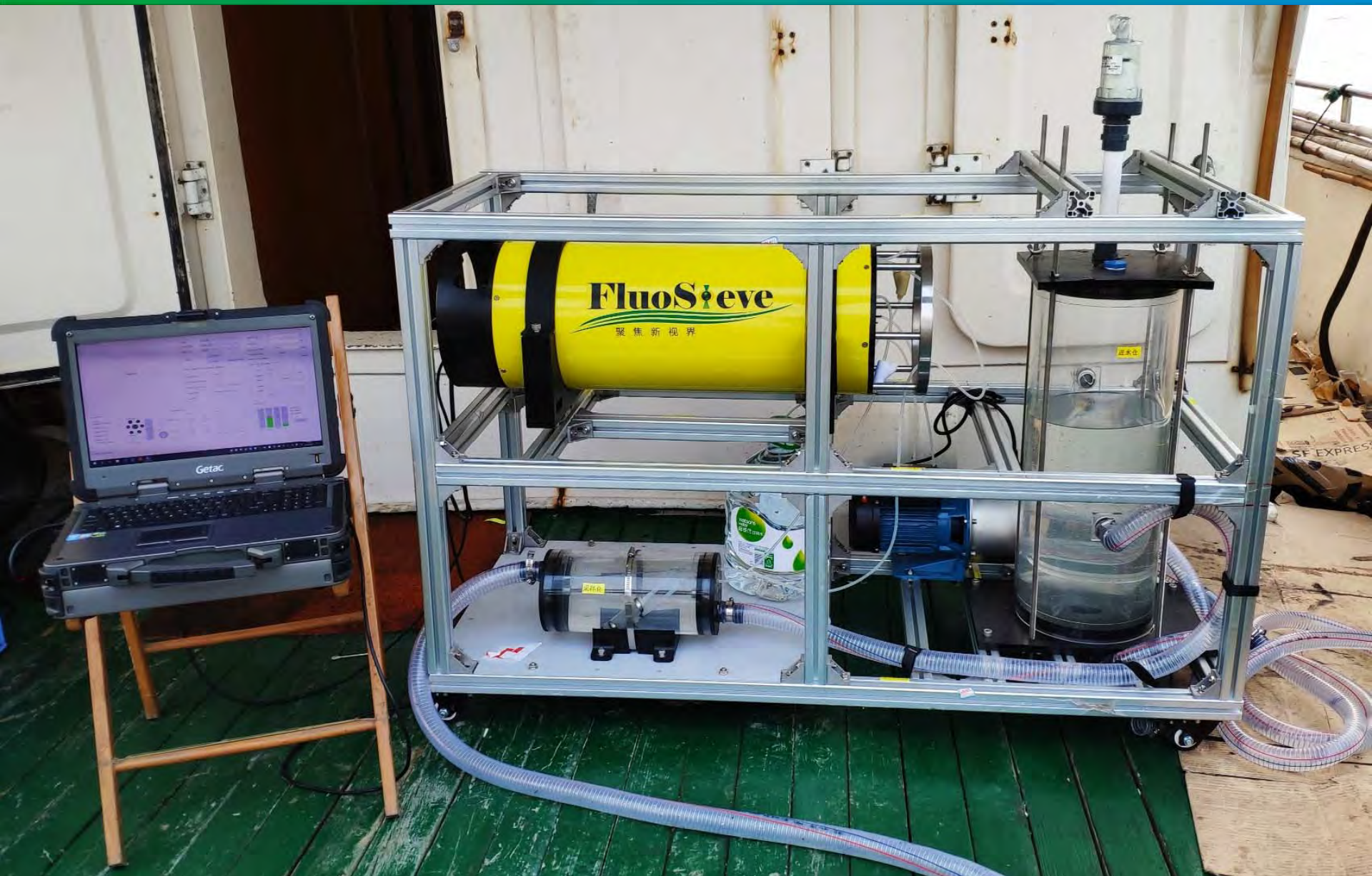
Sea Test on Boat



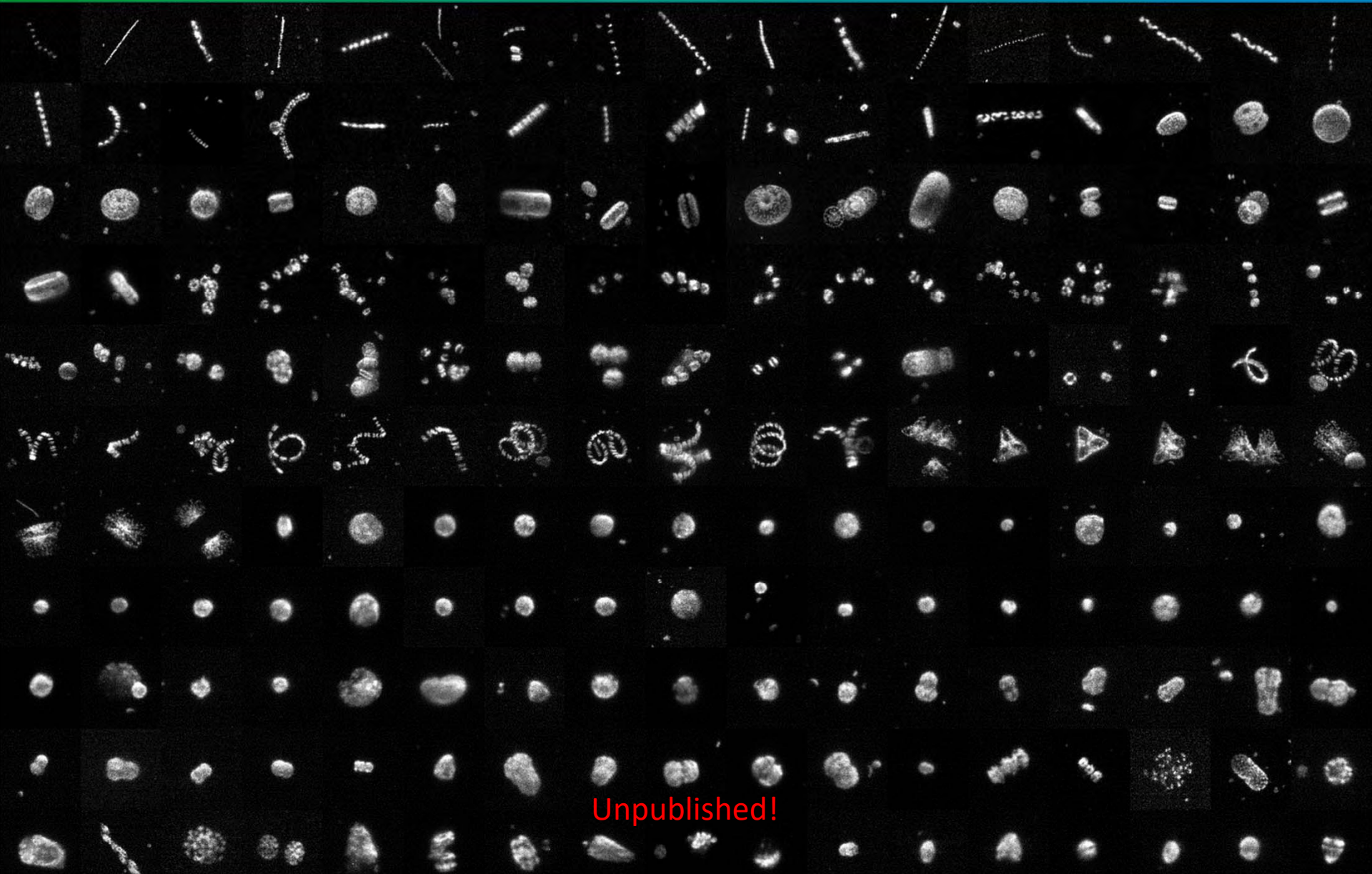
- North latitude 24.425010
  East longitude 118.100188

Sea Test inside the Boat Lab

Sea Test on the Boat Deck



First Images of Natural Sample



How can IFCs serve OCS?

Current Limitations

- 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
- Identification of small cells (picoplankton) to species level difficult
- Sensitive to methods used to collect and concentrate cells
- Preservation techniques do not work equally well for all taxa
- 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

IFC Capability or Potential

- ✓ High-throughput and more
- ✓ HI+AI! Develop AI asap
- ✓ Further study with increase in analysis throughput, which is obviously a plus
- ✓ TDI-IFC and LSF-IFC can do
- ✓ Developing MCF-IFC and combine with molecular methods
- ✓ Developing field deployable IFCs with minimum water sample pretreatment
- ✓ LSF-IFC already enlarged sizing range, further enhance IFC sensitivity, resolution and FOV
- ✓ IFC provides morphological details and molecular specificity
- ✓ Manual work could be more expensive
- ✓ Better UI design and training, attract talents

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梦想成就未来 应用创造价值
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THANK YOU FOR YOUR ATTENTION!