Lecture 3:
Tonight's Assignment: An introduction to phytoplankton in Harpswell Sound using the Imaging Flow CytoBot

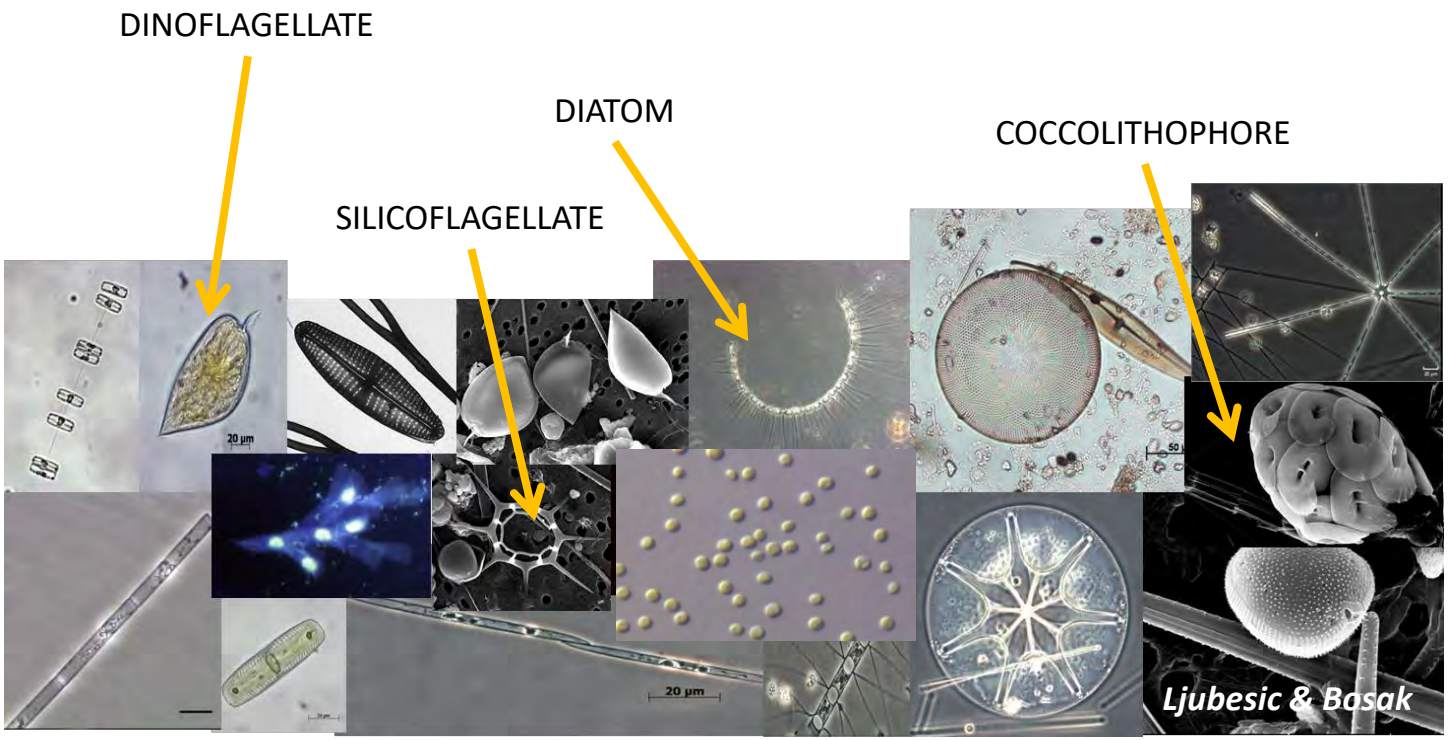
What are phytoplankton?

IVONA CETINIĆ

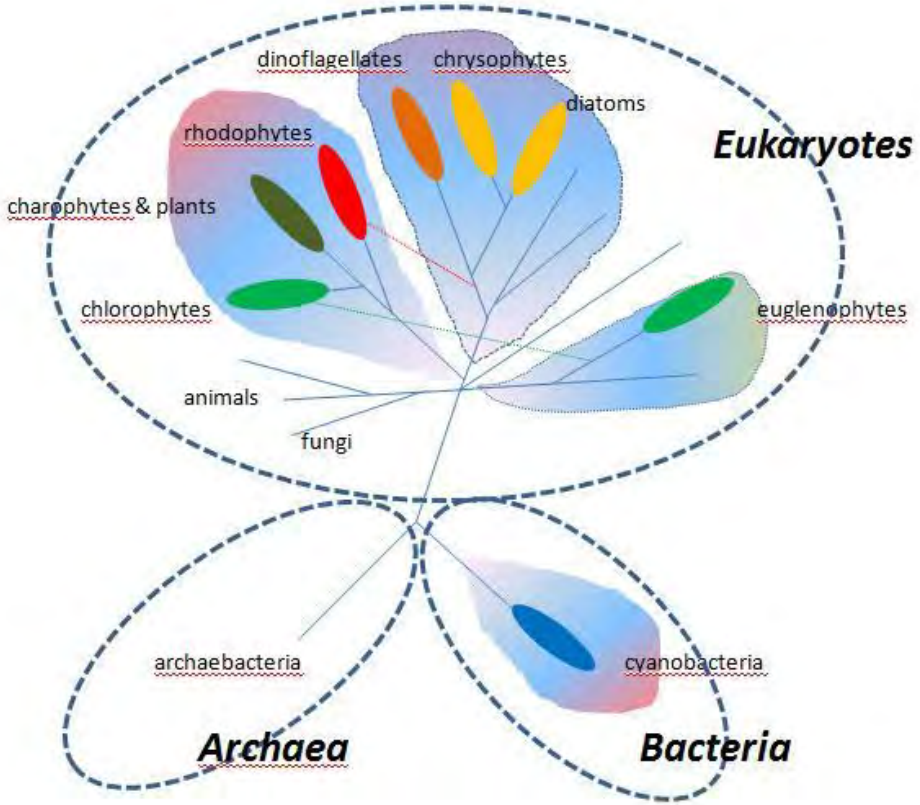
NASA GSFC / USRA

@teuta

PHYTOPLANKTON
"drifting plants", 1887



GREAT GENETIC DIVERSITY OF ORGANISMS THAT INTERACT WITH LIGHT IN THE OCEAN



Keeling et al. 2004
http://www.diatom.org

PHYTO

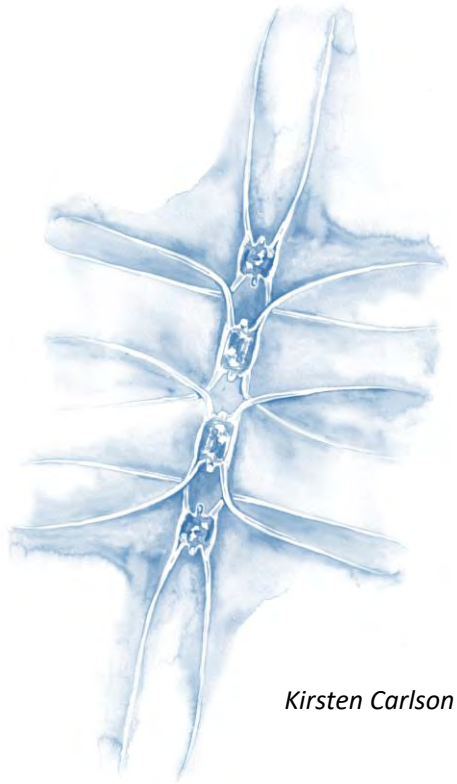
process of photosynthesis:

DRAW DOWN CARBON DIOXIDE

MAKE CARBOHYDRATES

(BASE OF MARINE FOOD WEB)

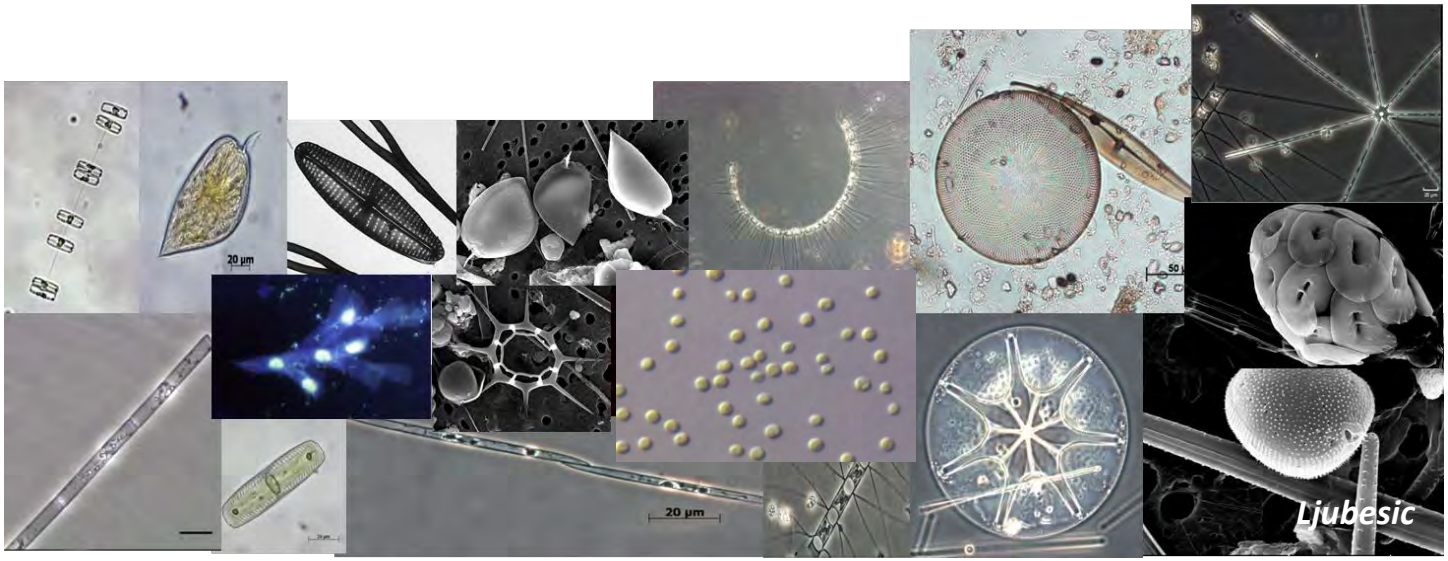
PRODUCE OXYGEN



Kirsten Carlson SOI

$$6CO_2 \text{ Carbon dioxide} + 6H_2O \text{ Water} \xrightarrow{\text{Light}} C_6H_{12}O_6 \text{ Sugar} + 6O_2 \text{ Oxygen}$$

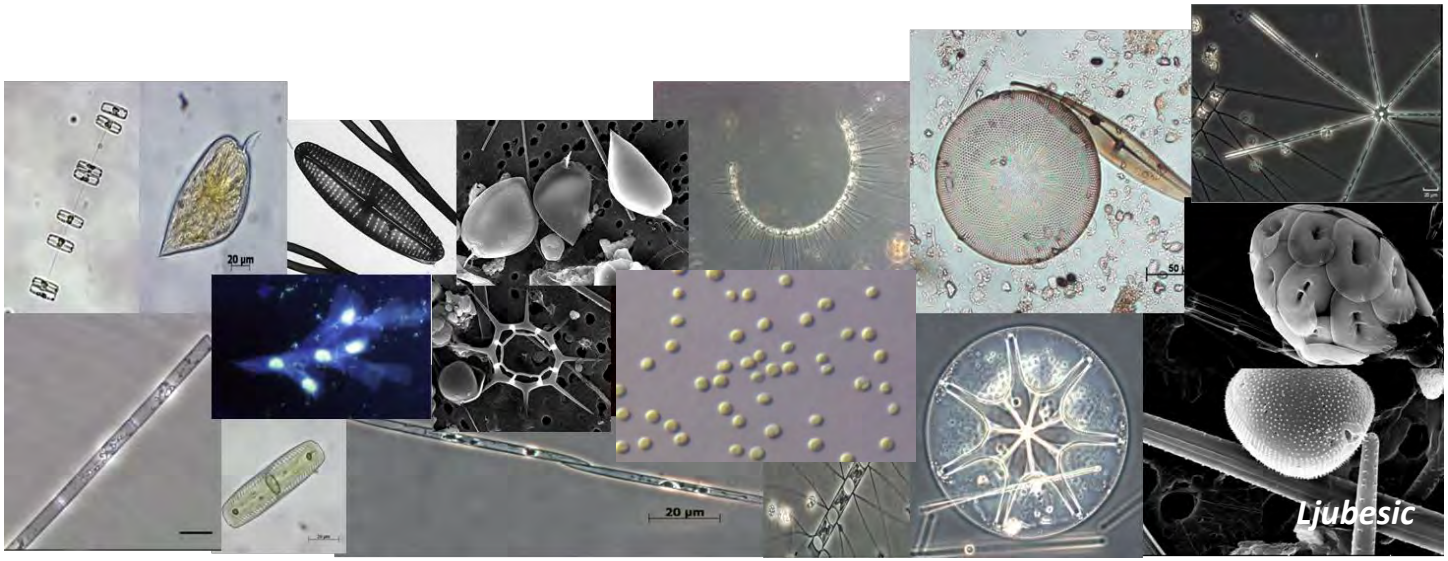
PHYTOPLANKTON
Many shapes, colors and sizes..
…that define their role in marine ecosystem and oceanic elemental cycles



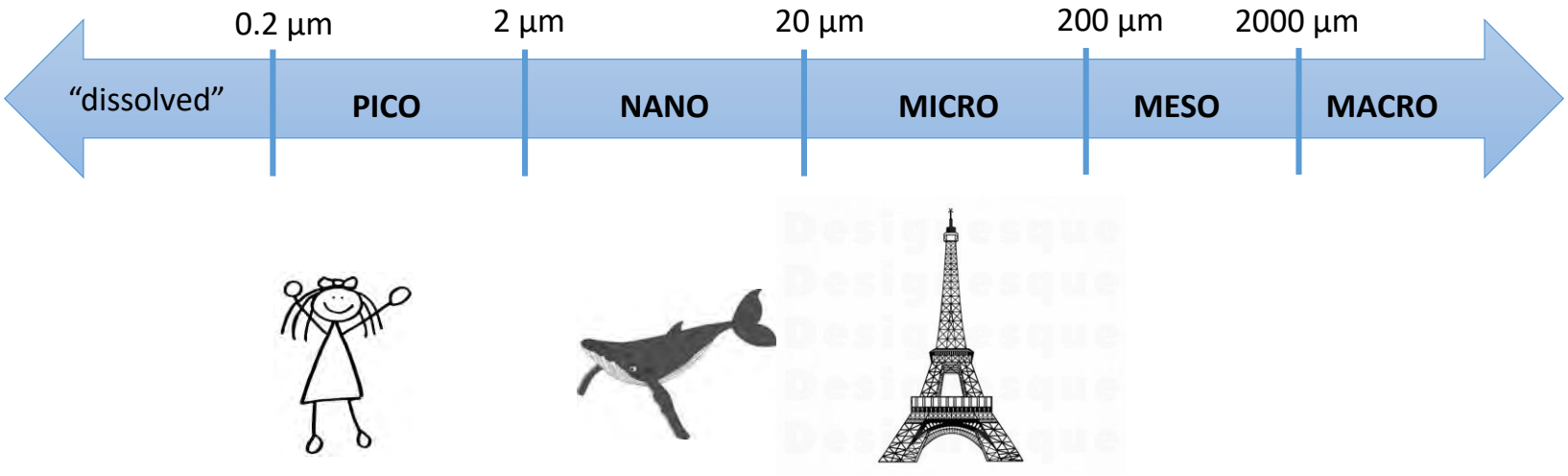
PHYTOPLANKTON

Many shapes, colors and sizes..

…that define their optical signal

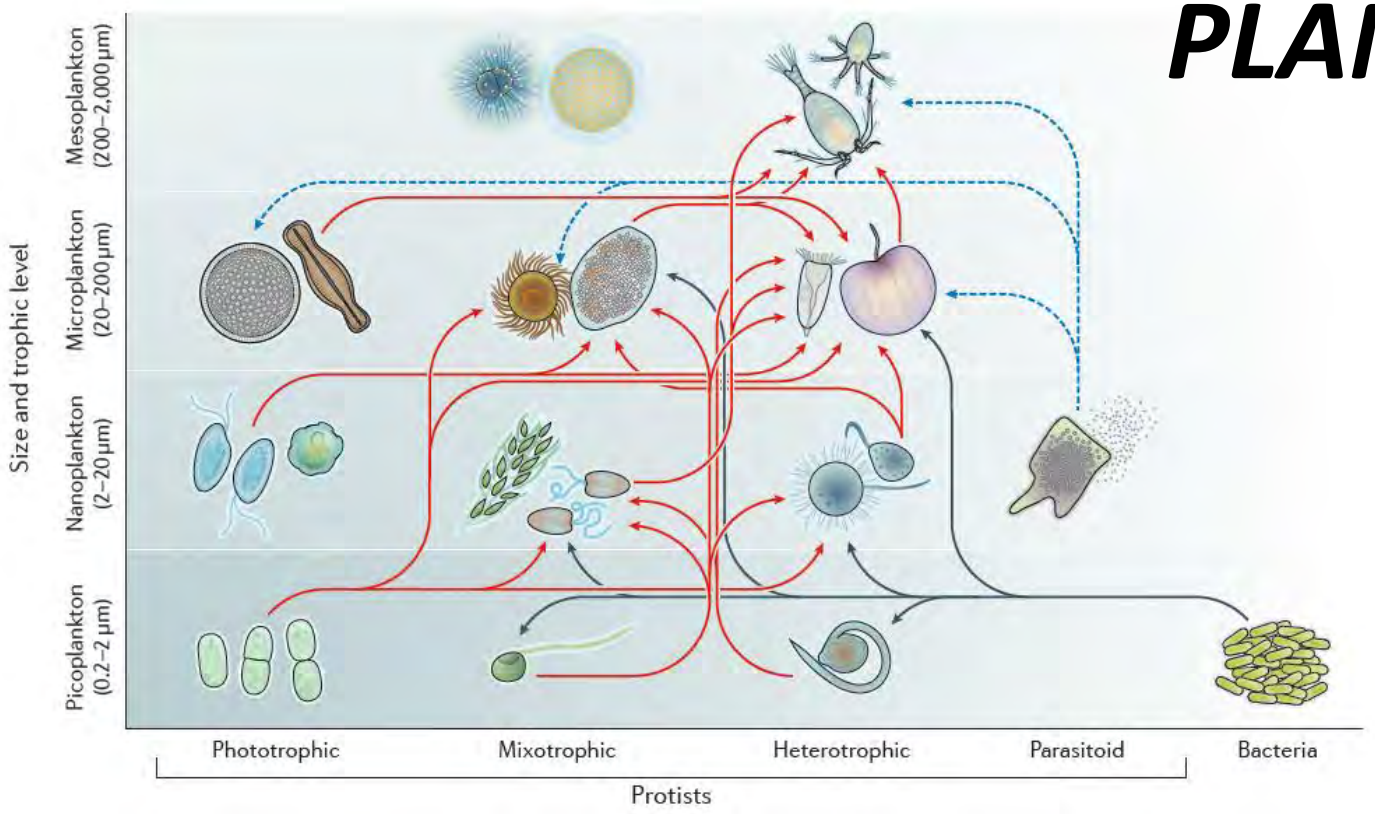


PHYTOPLANKTON AS PARTICLES



Le Quéré et al. (2005)

PLANKTON



Caron et al, 2017

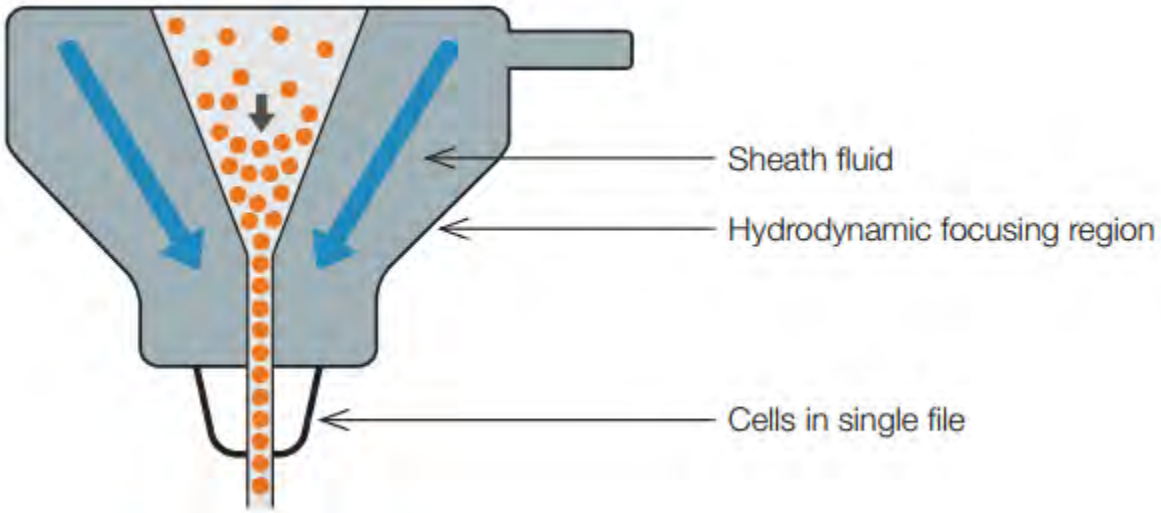
- Who they are (classification)
  - Size
  - Biogeochemical role

Flow Cytobot (FCB)

- Heidi Sosik and Rob Olsen, WHOI
  - Started with benchtop flow cytometer

Flow cytometry basics

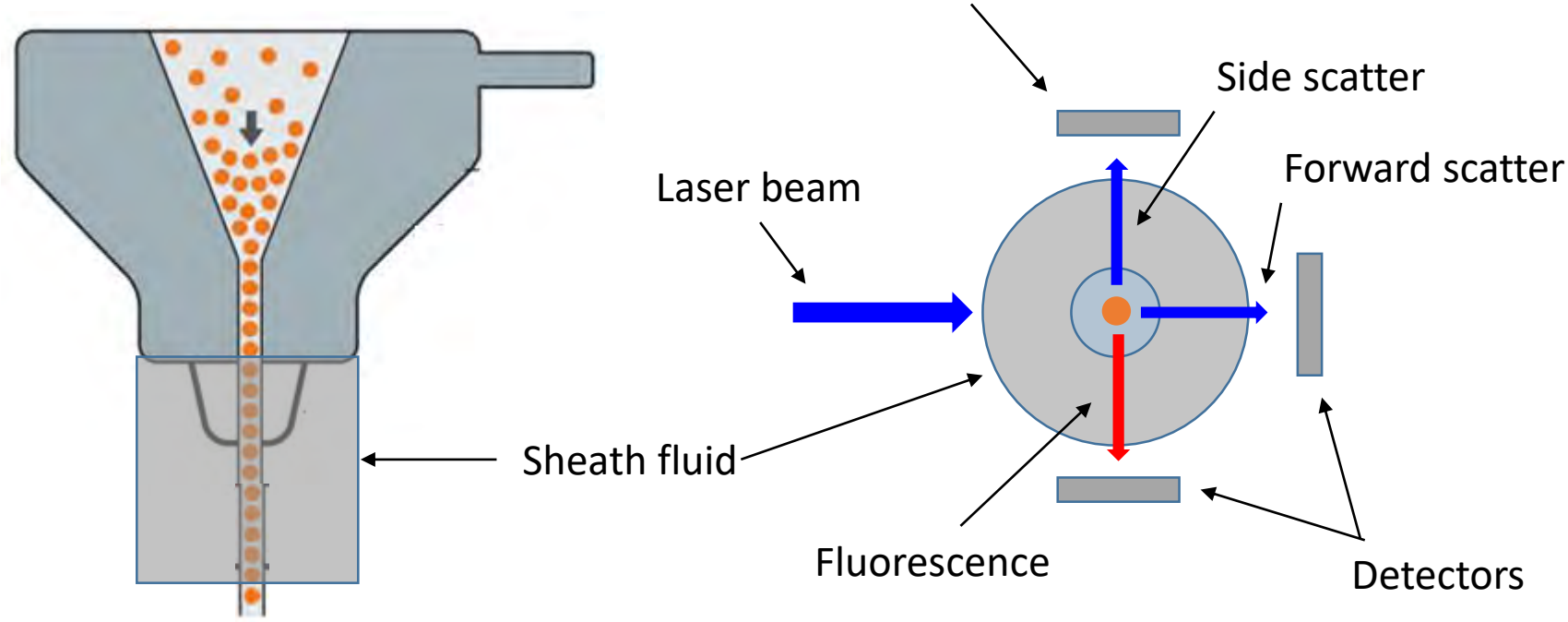
- Hydrodynamic focusing (single cell line up)



https://www.bio-rad-antibodies.com/flow-cytometry-fluidics-system.html

Flow cytometry basics

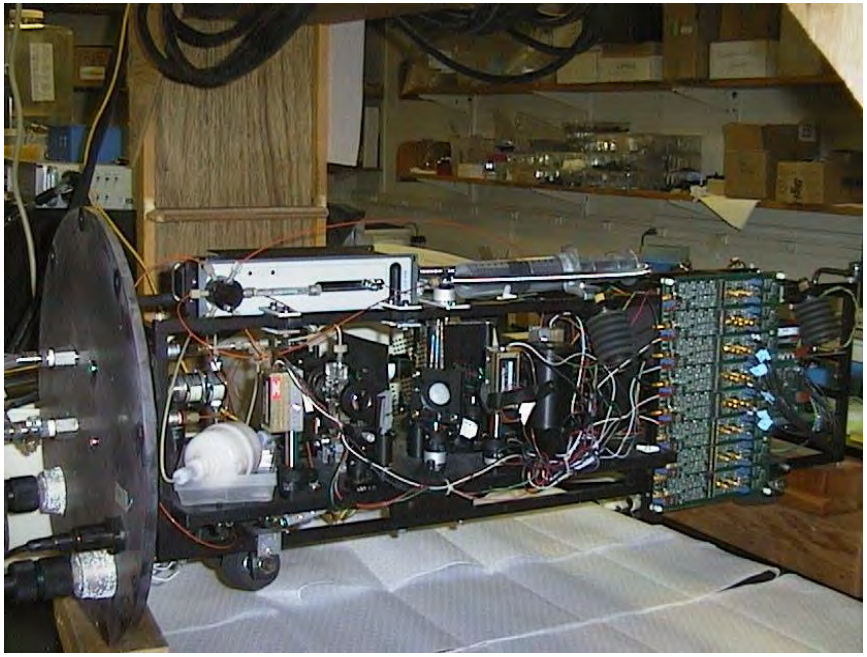
- Optical interrogation (lasers and detectors)



https://www.bio-rad-antibodies.com/flow-cytometry-fluidics-system.html

Flow Cytobot (FCB)

- Heidi Sosik and Rob Olsen, WHOI
  - Started with benchtop flow cytometer
  - Optimized it for phytoplankton (lasers)
  - Made it submersible

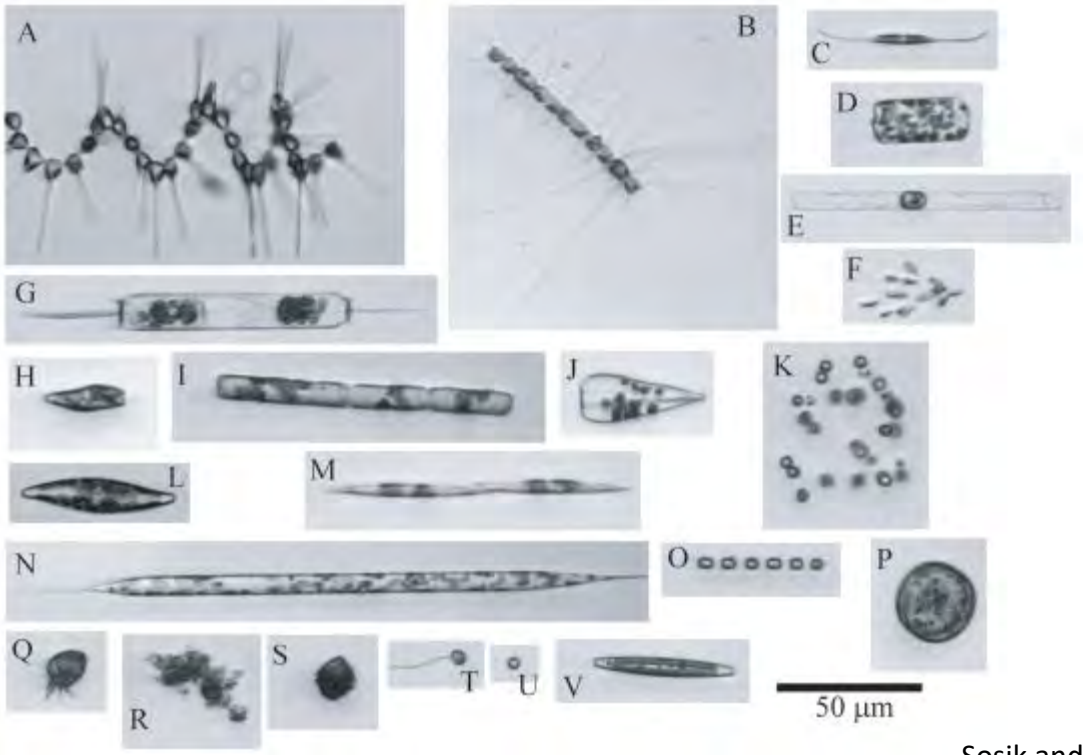


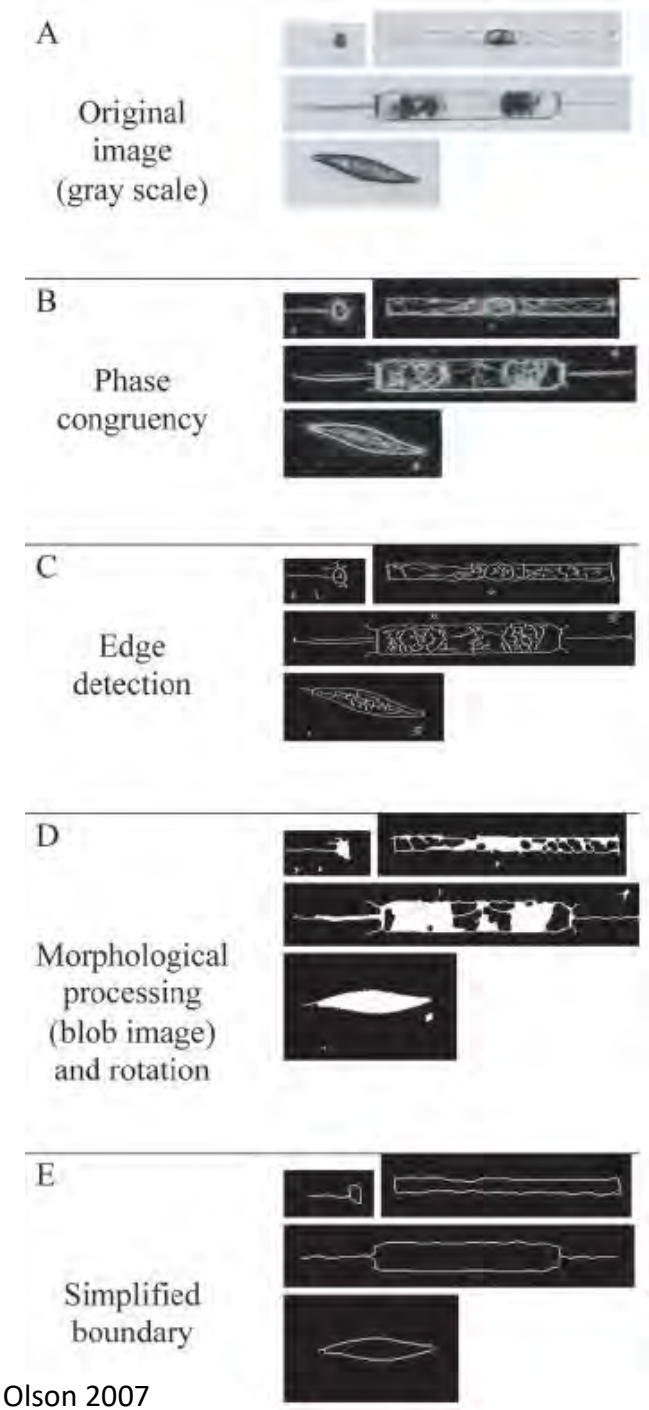
Imaging Flow Cytobot (IFCB)

- Heidi Sosik and Rob Olsen, WHOI
  - Started with benchtop flow cytometer
  - Optimized it for phytoplankton (lasers)
  - Made it submersible
  - Used fluorescence and/or scattering signal from individual cell to trigger CCD camera imaging

IFCB

- Images of cells
- Blob processing
- Feature processing

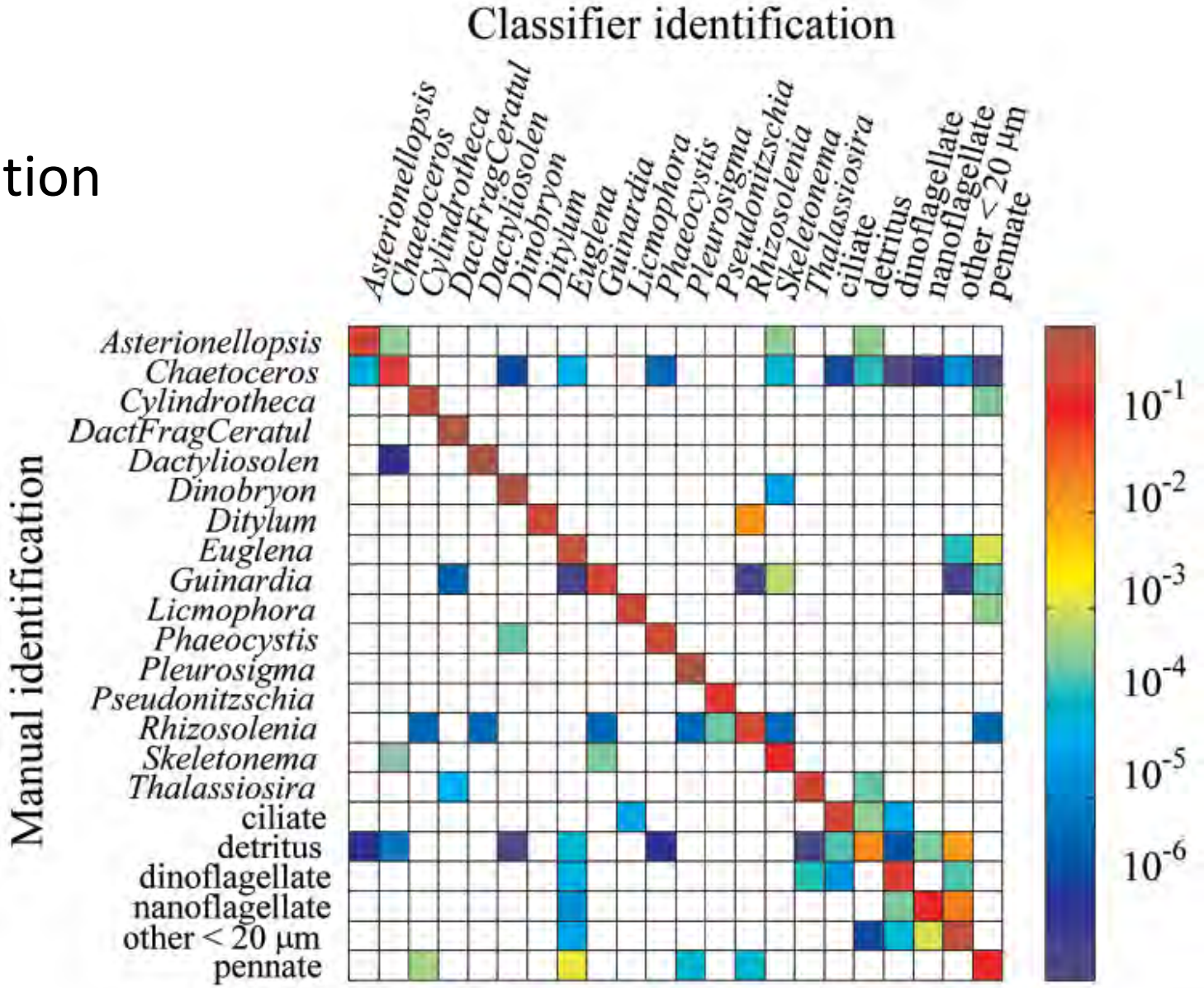




Sosik and Olson 2007

IFCB

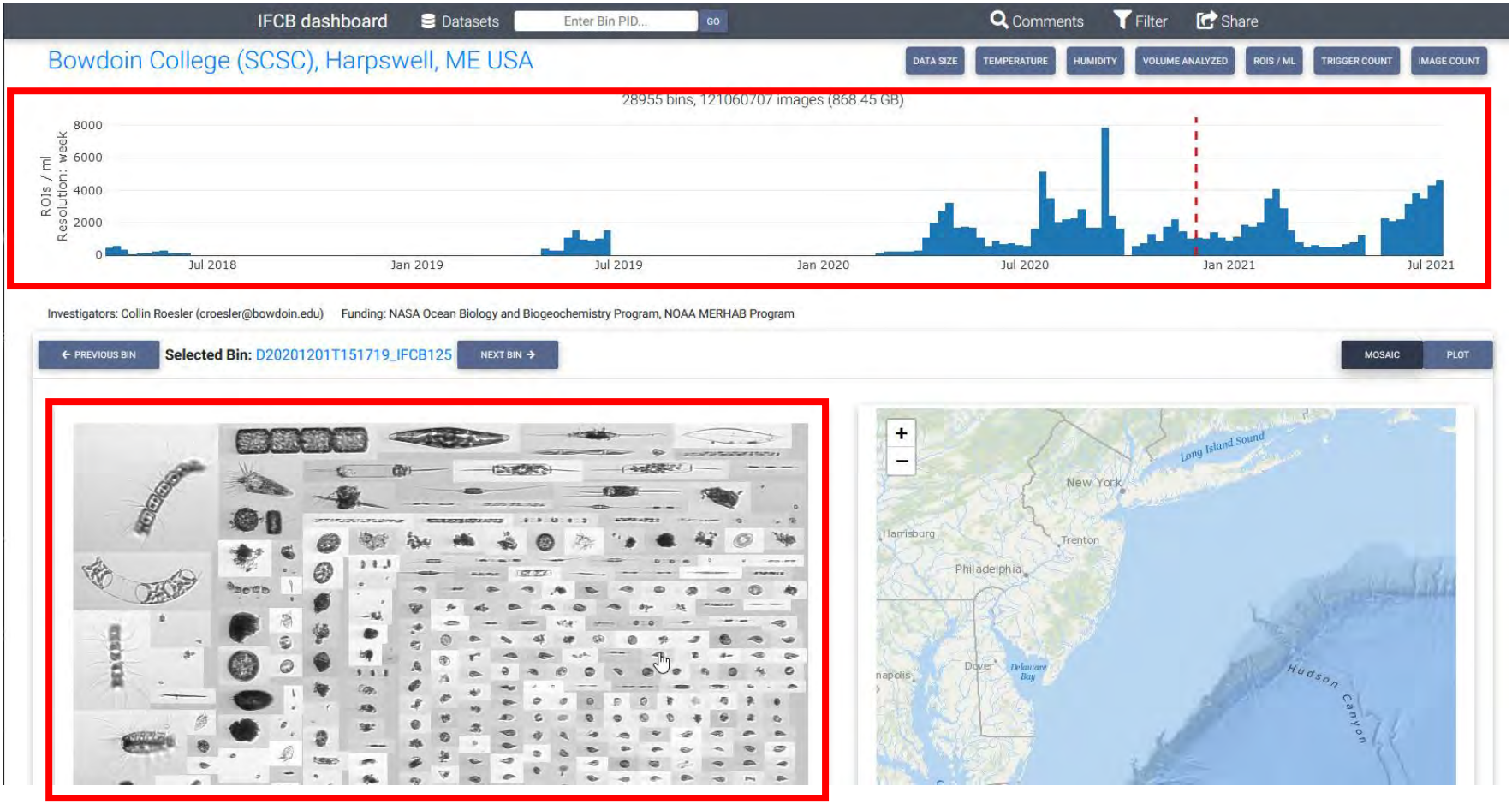
- classification



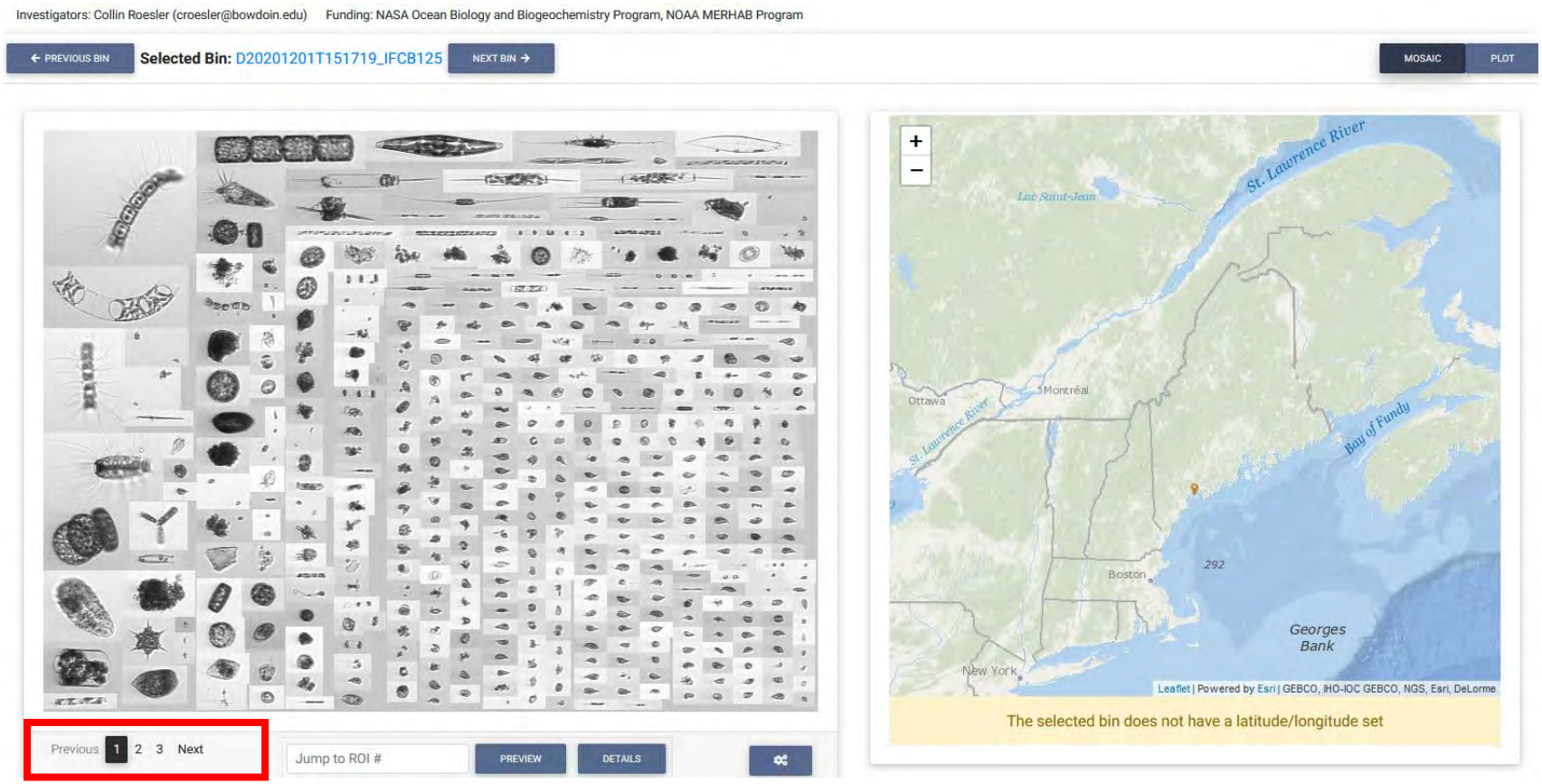
Sosik and Olson 2007

Data are shared via a dashboard

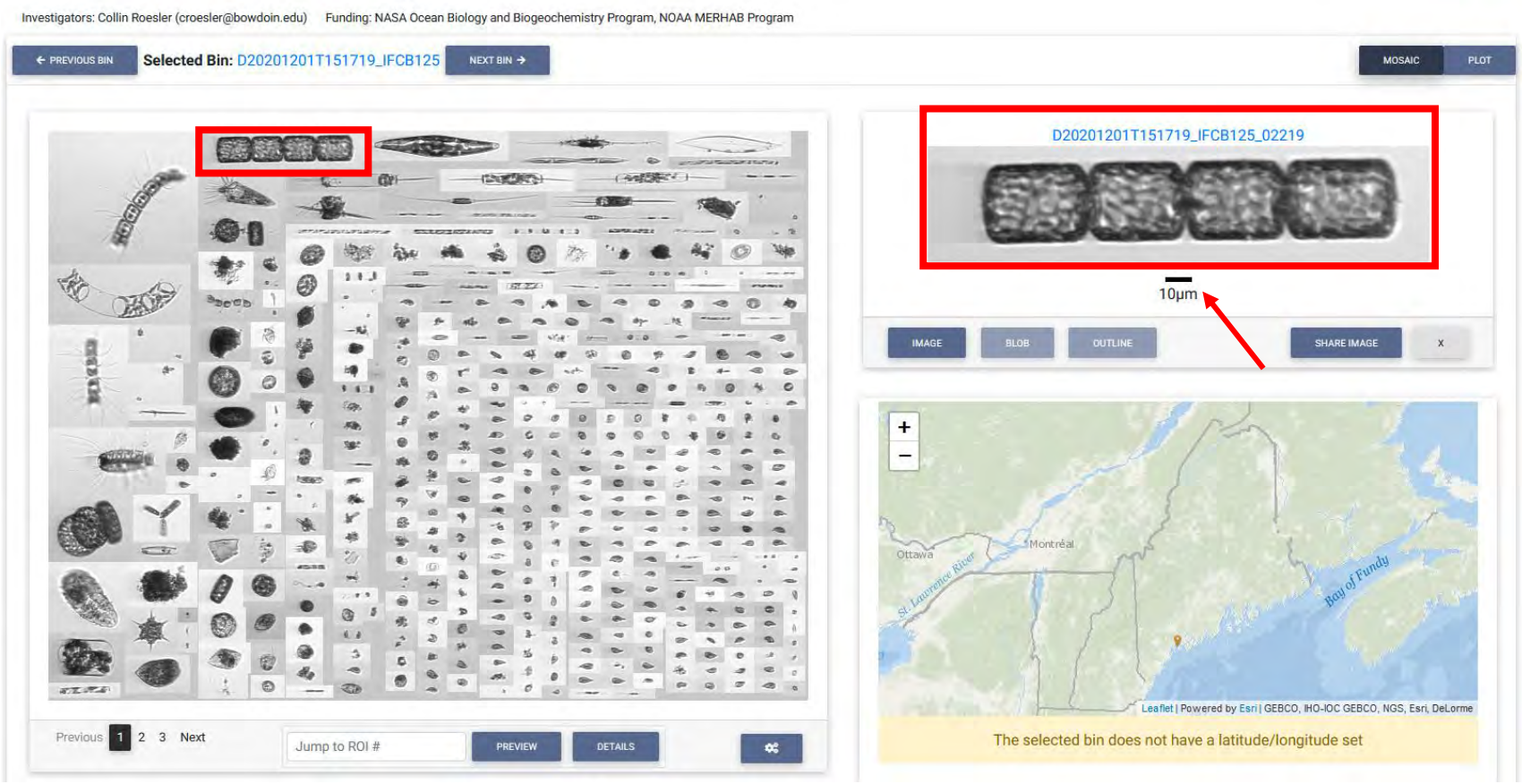
- https://ifcb-data.whoi.edu/timeline?dataset=harpswell&bin=D20201201T151719_IFCB125



Mosaic page 1/3 and location map

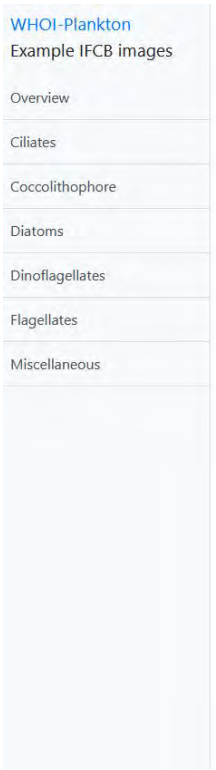


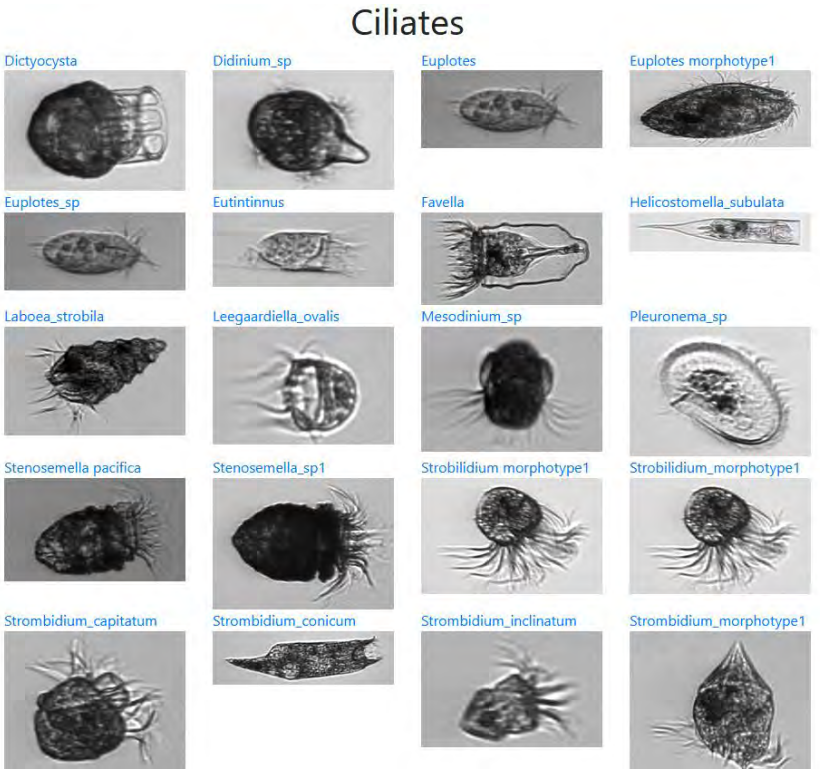
Click on any roi (region of interest)



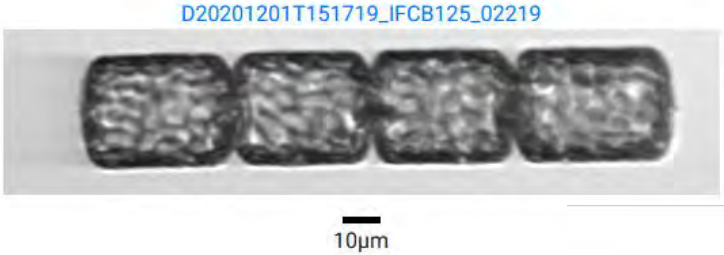
Examples of images for taxonomic classification

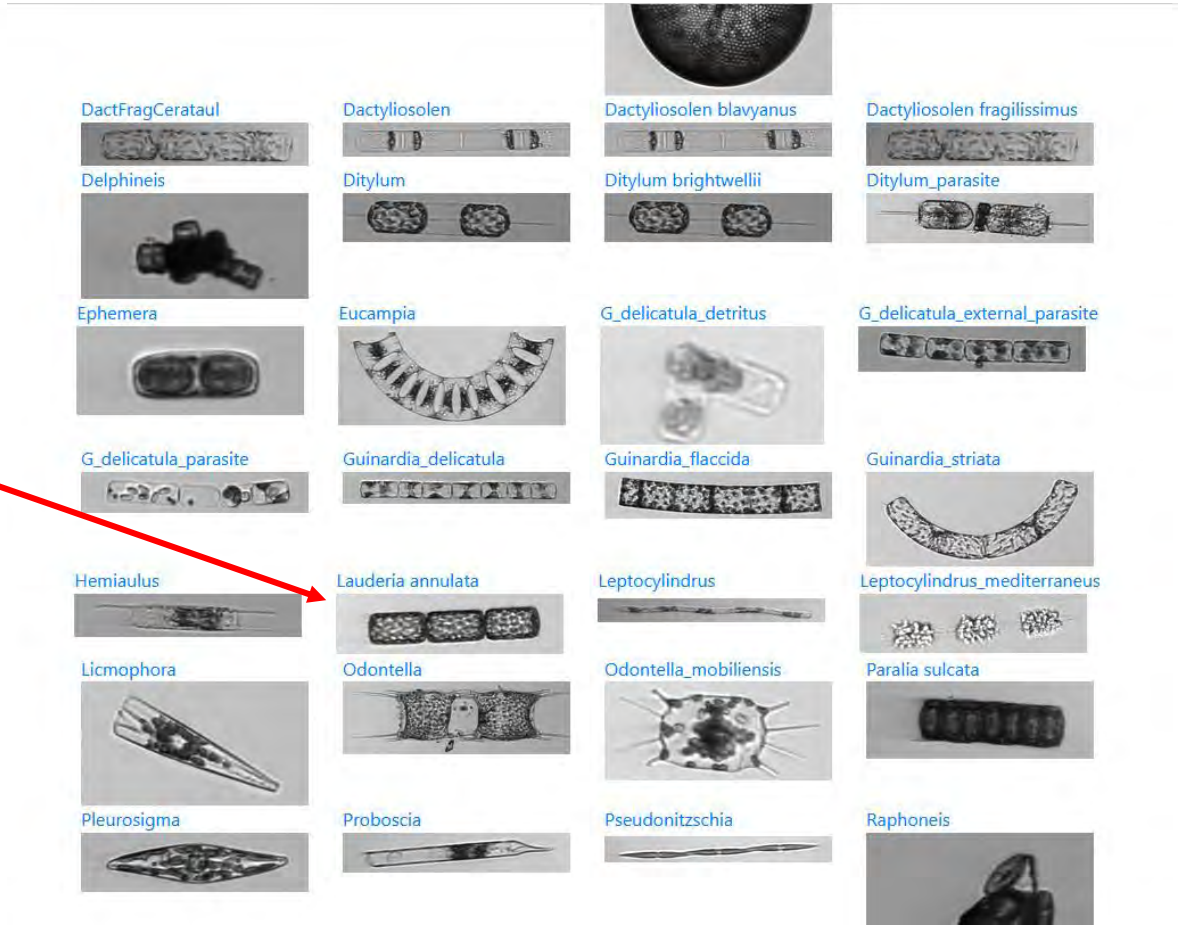
- https://whoigit.github.io/whoi-plankton/index.html





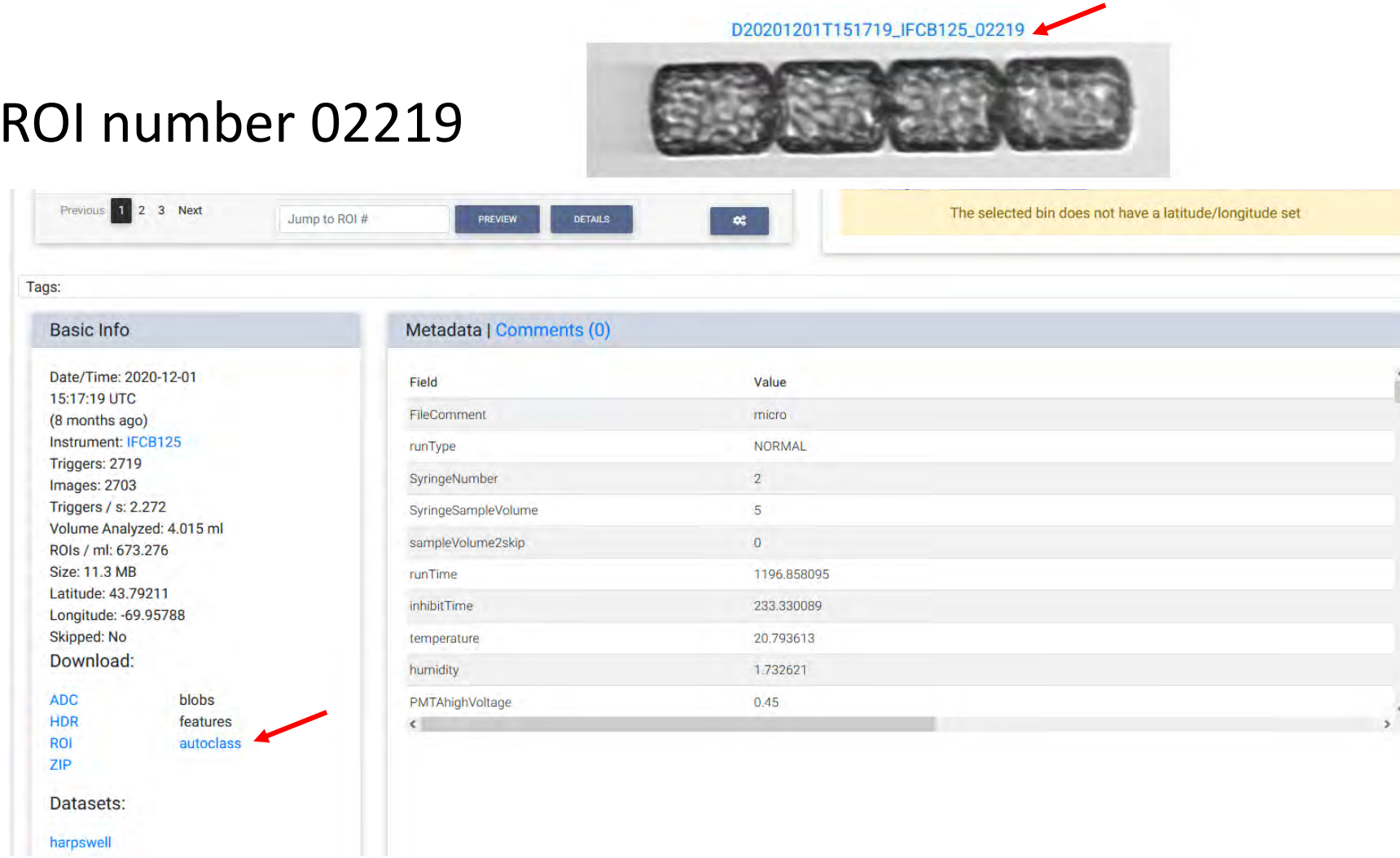
Example to identify



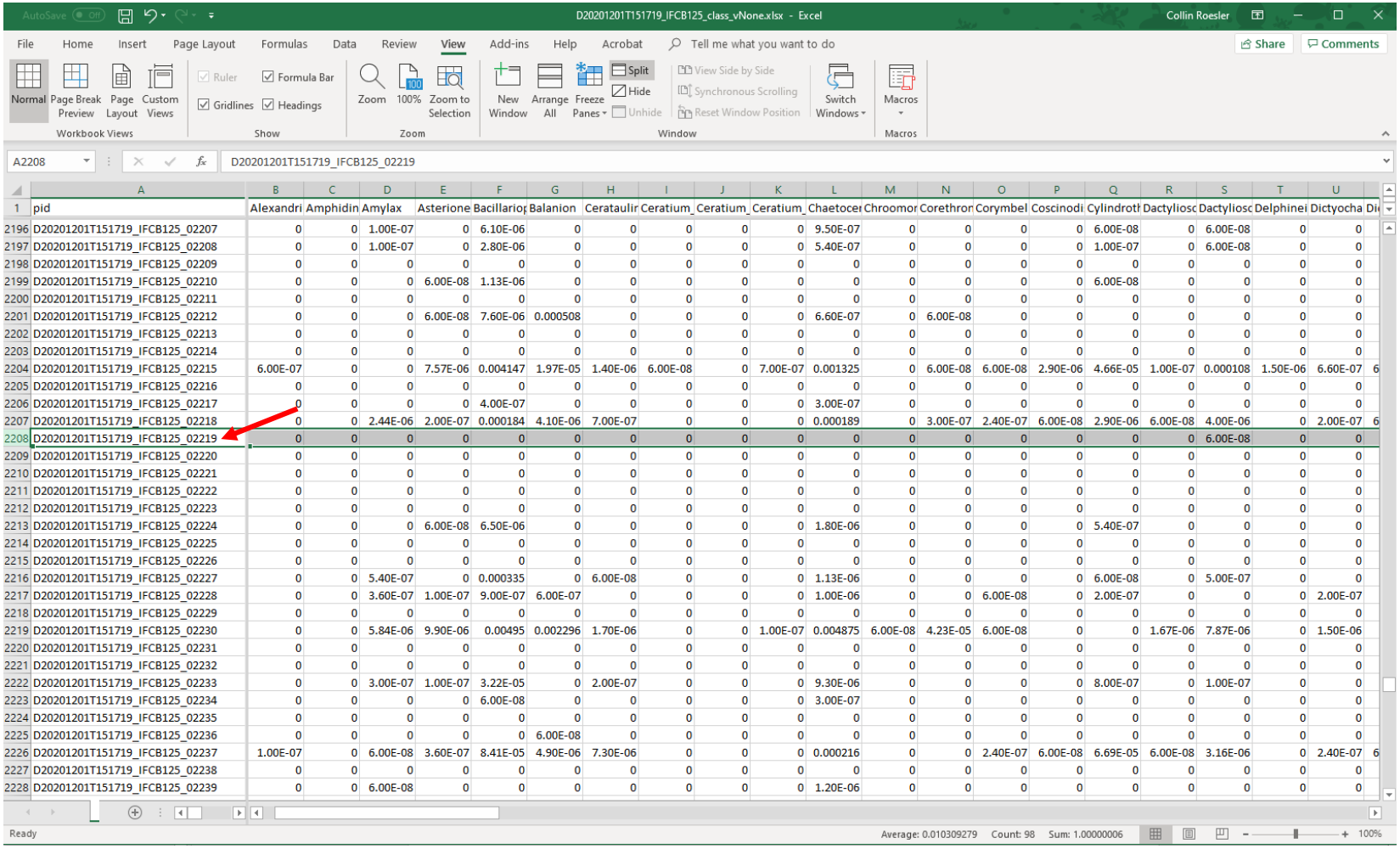


Check automated classification

- ROI number 02219



Download automated classification file. Find ROI row, look at *probabilities*



D20201201T151719_IFCB125_class_vNone.xlsx - Excel

A2208 D20201201T151719_IFCB125_02219

	pid	Alexandri	Amphidin	Amylax	Asterione	Bacillario	Balanion	Cerataulir	Ceratium_	Ceratium_	Ceratium_	Chaetocer	Chroomor	Corethron	Corymbel	Coscinodi	Cylindrotl	Dactyliosc	Dactyliosc	Delphinei	Dictyocha	Di
2196	D20201201T151719_IFCB125_02207	0	0	1.00E-07	0	6.10E-06	0	0	0	0	0	9.50E-07	0	0	0	0	6.00E-08	0	6.00E-08	0	0	
2197	D20201201T151719_IFCB125_02208	0	0	1.00E-07	0	2.80E-06	0	0	0	0	0	5.40E-07	0	0	0	0	1.00E-07	0	6.00E-08	0	0	
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2199	D20201201T151719_IFCB125_02210	0	0	0	6.00E-08	1.13E-06	0	0	0	0	0	0	0	0	0	0	6.00E-08	0	0	0	0	
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2205	D20201201T151719_IFCB125_02216	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2206	D20201201T151719_IFCB125_02217	0	0	0	0	4.00E-07	0	0	0	0	0	3.00E-07	0	0	0	0	0	0	0	0	0	
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2216	D20201201T151719_IFCB125_02227	0	0	5.40E-07	0	0.000335	0	6.00E-08	0	0	0	1.13E-06	0	0	0	0	6.00E-08	0	5.00E-07	0	0	
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Ready Average: 0.010309279 Count: 98 Sum: 1.00000006 100%

Download classification file find roi row, look at probabilities

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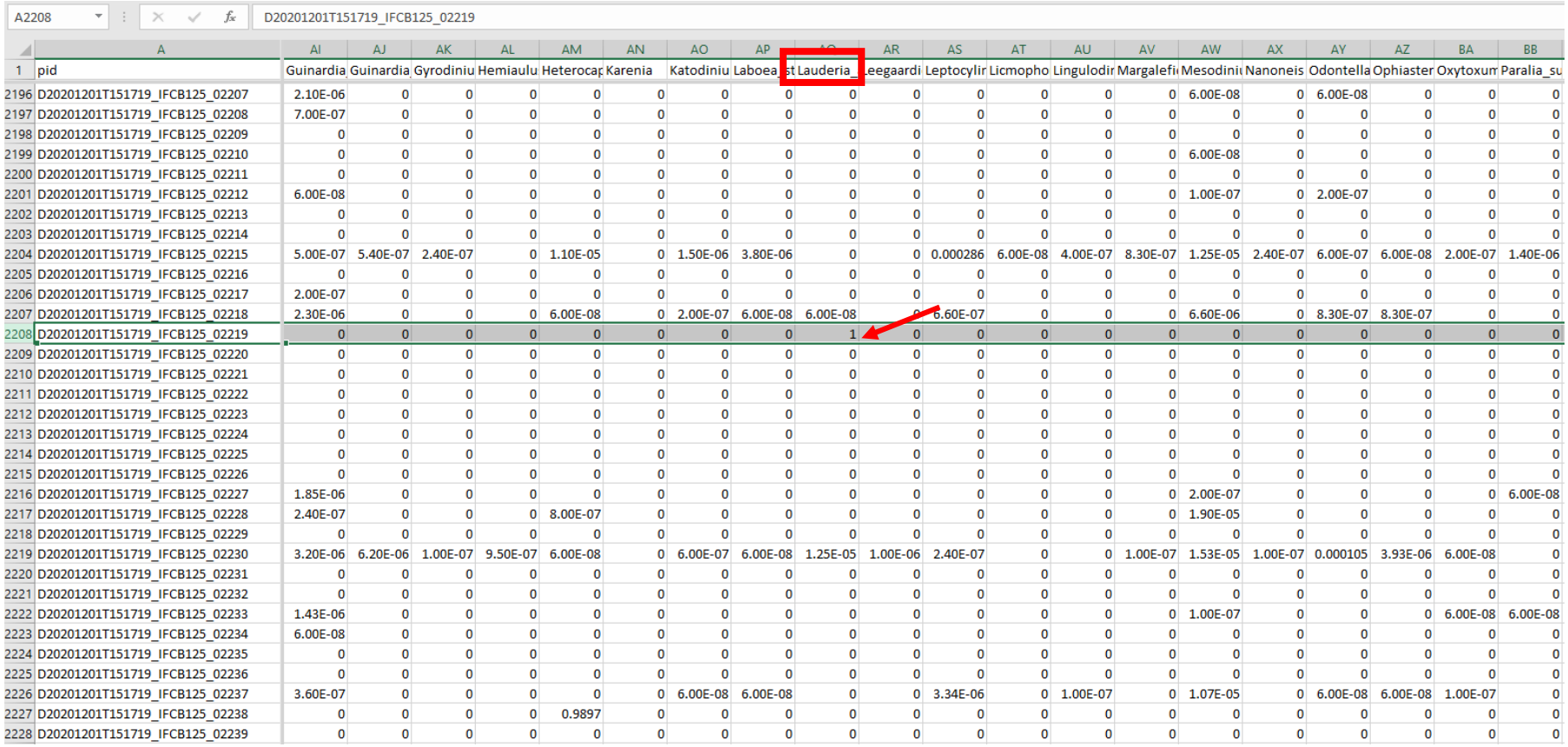

A2208 | D20201201T151719_IFCB125_02219

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1	pid	Guinardia	Guinardia	Gyrodiniu	Hemiaulu	Heterocap	Karenia	Katodiniu	Laboea_st	Lauderia_	Leegaardi	Leptocylir	Licmopho	Linguloдir	Margalefi	Mesodinii	Nanoneis	Odontella	Ophiaster	Oxytoxum	Paralia_su
2196	D20201201T151719_IFCB125_02207	2.10E-06	0	0	0	0	0	0	0	0	0	0	0	0	0	6.00E-08	0	6.00E-08	0	0	0
2197	D20201201T151719_IFCB125_02208	7.00E-07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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2202	D20201201T151719_IFCB125_02213	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2203	D20201201T151719_IFCB125_02214	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2204	D20201201T151719_IFCB125_02215	5.00E-07	5.40E-07	2.40E-07	0	1.10E-05	0	1.50E-06	3.80E-06	0	0	0.000286	6.00E-08	4.00E-07	8.30E-07	1.25E-05	2.40E-07	6.00E-07	6.00E-08	2.00E-07	1.40E-06
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2211	D20201201T151719_IFCB125_02222	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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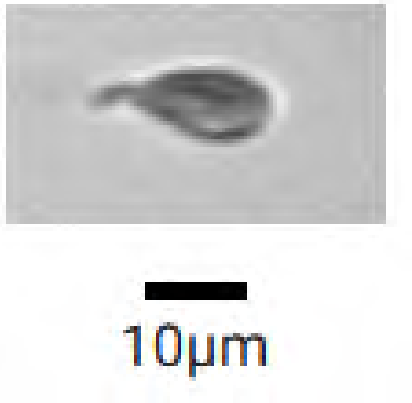
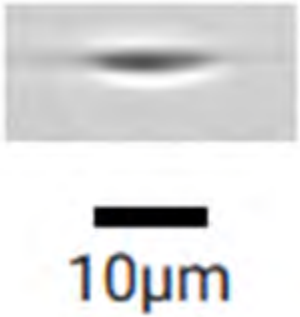
2215	D20201201T151719_IFCB125_02226	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2216	D20201201T151719_IFCB125_02227	1.85E-06	0	0	0	0	0	0	0	0	0	0	0	0	0	2.00E-07	0	0	0	0	6.00E-08
2217	D20201201T151719_IFCB125_02228	2.40E-07	0	0	0	8.00E-07	0	0	0	0	0	0	0	0	0	1.90E-05	0	0	0	0	0
2218	D20201201T151719_IFCB125_02229	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2219	D20201201T151719_IFCB125_02230	3.20E-06	6.20E-06	1.00E-07	9.50E-07	6.00E-08	0	6.00E-07	6.00E-08	1.25E-05	1.00E-06	2.40E-07	0	0	1.00E-07	1.53E-05	1.00E-07	0.000105	3.93E-06	6.00E-08	0
2220	D20201201T151719_IFCB125_02231	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2221	D20201201T151719_IFCB125_02232	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2222	D20201201T151719_IFCB125_02233	1.43E-06	0	0	0	0	0	0	0	0	0	0	0	0	0	1.00E-07	0	0	0	6.00E-08	6.00E-08
2223	D20201201T151719_IFCB125_02234	6.00E-08	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2224	D20201201T151719_IFCB125_02235	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2225	D20201201T151719_IFCB125_02236	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2226	D20201201T151719_IFCB125_02237	3.60E-07	0	0	0	0	0	6.00E-08	6.00E-08	0	0	3.34E-06	0	1.00E-07	0	1.07E-05	0	6.00E-08	6.00E-08	1.00E-07	0
2227	D20201201T151719_IFCB125_02238	0	0	0	0	0.9897	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2228	D20201201T151719_IFCB125_02239	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Test… we see a lot of these small but identifiable cells

- Cryptomonad (flagellates)



- Pennate diatom



Your challenge

- Work in 8 groups of 2 or 3
- Each group will receive the mosaic pages for a specific sample (one per month December – July)
- Construct a taxonomic histogram
  - Cut out the images
  - Sort by recognizable classification
  - Paste into histograms
  - See Lab 1.5 assignment for details
- We will discuss these tomorrow morning

Specifics

- Each group will receive a print out of all the images from a sample collected close to noon on the first day of the month starting in December 2020.
- Look through and begin to identify the most common species.
- Get out your scissors. Cut out the images and sort them into taxonomic groups based on their distinguishable features. For the very small cells that are difficult to identify, simply cut the mosaic into 2-inch strips. These will be “unclassified nanoplankton”.
- Once you have your images grouped by like classification, rank the groups from largest cells to smallest cells. Figure out how many groups you have been able to distinguish.
- Confer with the other groups to see if you all have identified the same groups. Once you have compared, determine the total number of phytoplankton groups across all the months, and rank them by size.
- Draw 2-inch “bins” along the x-axis of your poster paper. Label each bin on the x-axis with the complete list of phytoplankton groups, ranked smallest cells to largest (“unclassified nanoplankton” will be the first bin from left to right. ***Again, ensure each group has the same order of species on the x-axis, even if you don’t have any in your particular sample***.
- Tape your images into their bin along the x-axis.
- The result will be a histogram in the format of a size distribution.