May 2nd, 10:30 AM - 12:00 PM

Use of Flowcam Technology for Phytoplankton Monitoring in Central Puget Sound

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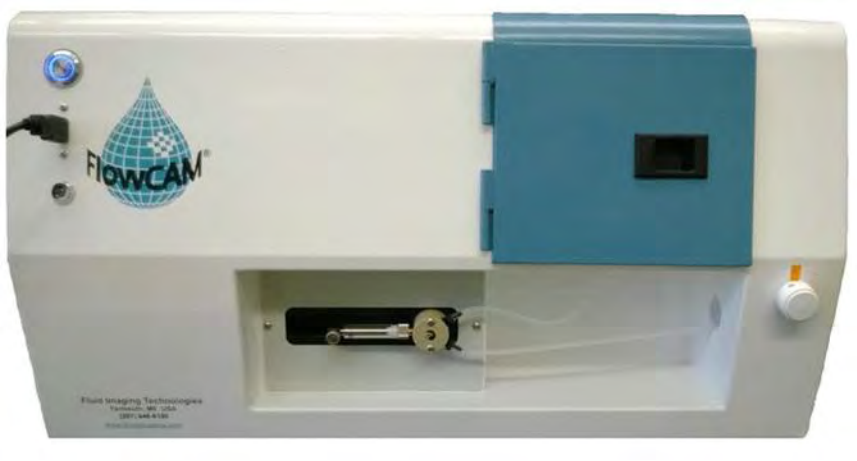
King County

What is FlowCAM?

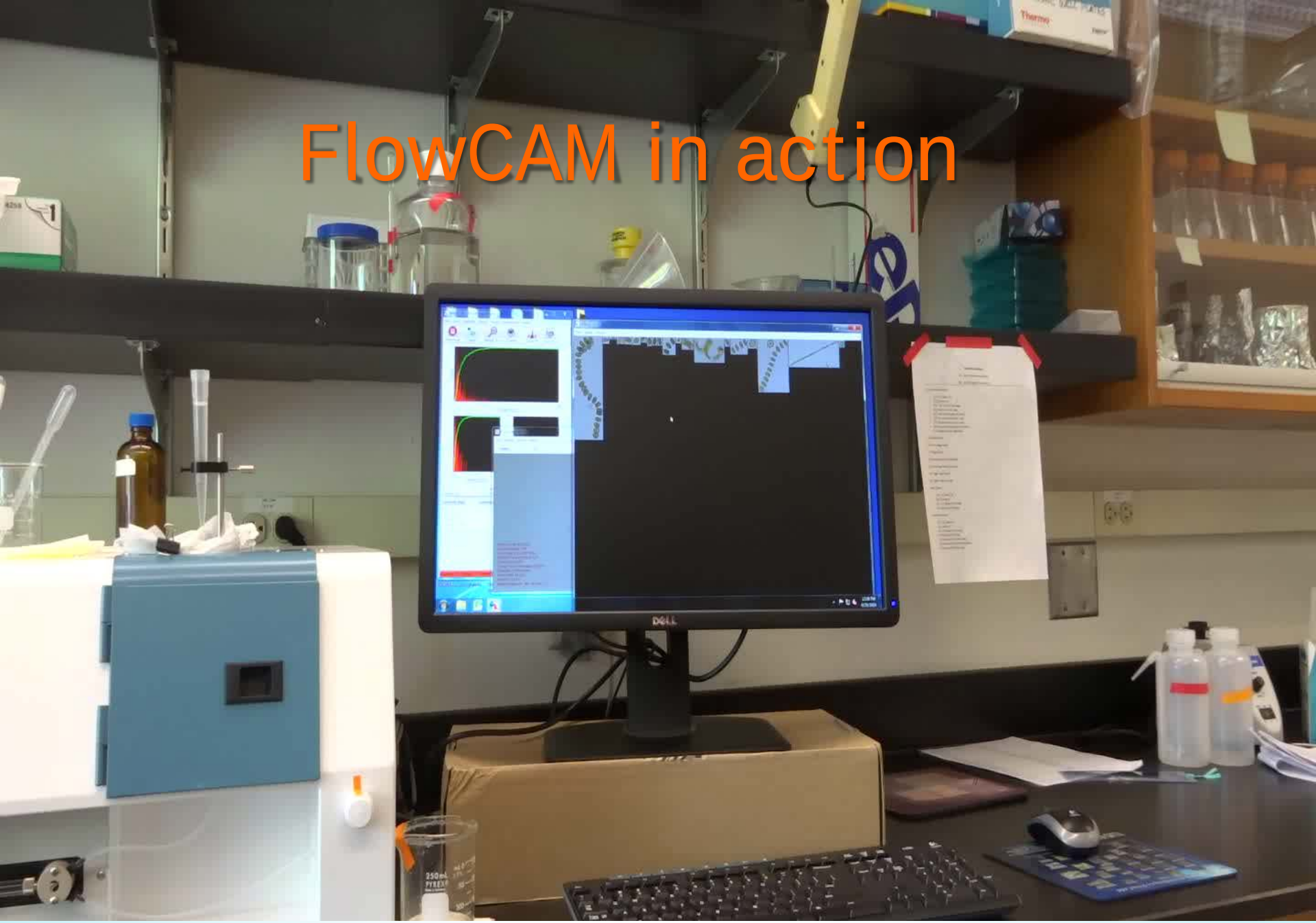
Imaging particle analyzer

- Flow cell + microscope + camera
  → each particle in field of view is imaged, analyzed, saved
  → single file contains all images from individual particles
- Pattern recognition software



FLUID IMAGING TECHNOLOGIES

FlowCAM in action



Why FlowCAM?

- Puget Sound phytoplankton: significant data gap
- Need ability to quantitatively characterize Puget Sound phytoplankton

- King County long term marine monitoring program
- Qualitative phytoplankton since 2008

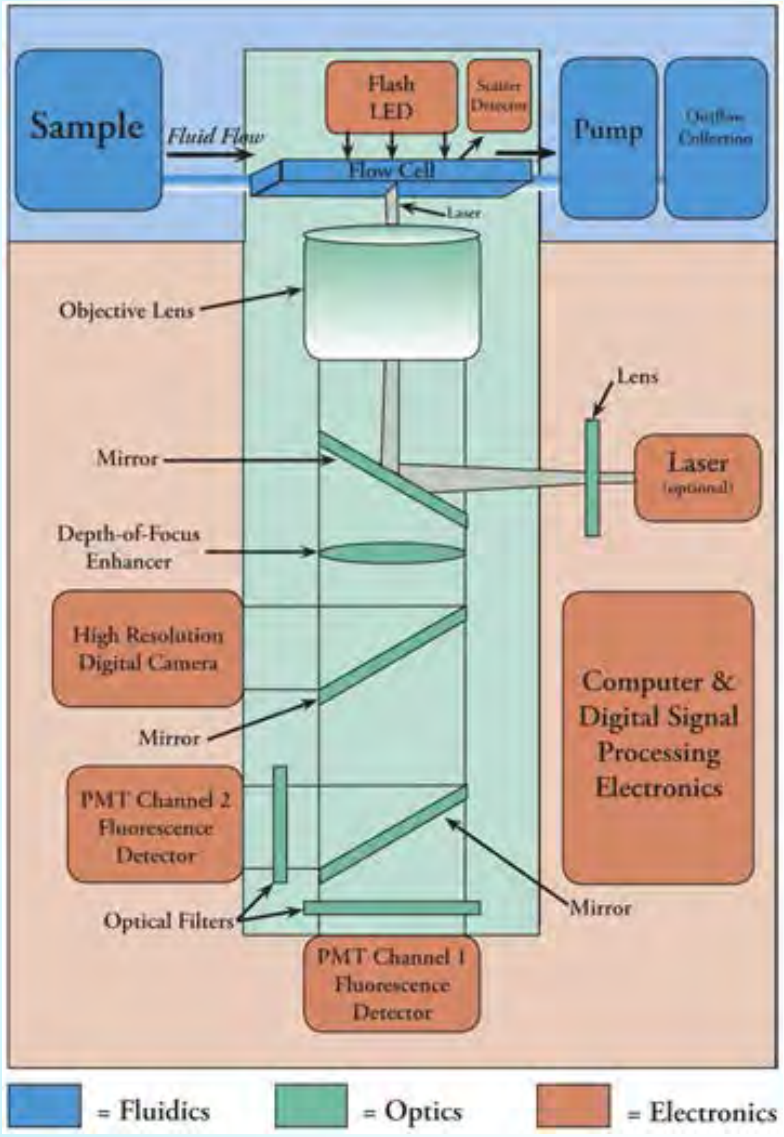
- FlowCAM:
  - Population changes over temporal and spatial scales
  - Interaction with multiple environmental variables (complex data)
  - Biodiversity/Biomass/Productivity
  - Trophic structure
  - Rapid identification and counts of HAB species

Sample Preparation

- Fresh live sample is best
- Minimal preparation
  - Filter to separate size fractions

    100 µm, 300 µm
  - Large size fraction using microscopy or FlowCAM at lower magnification
  - Dilution or concentration for certain samples

FlowCAM Hardware



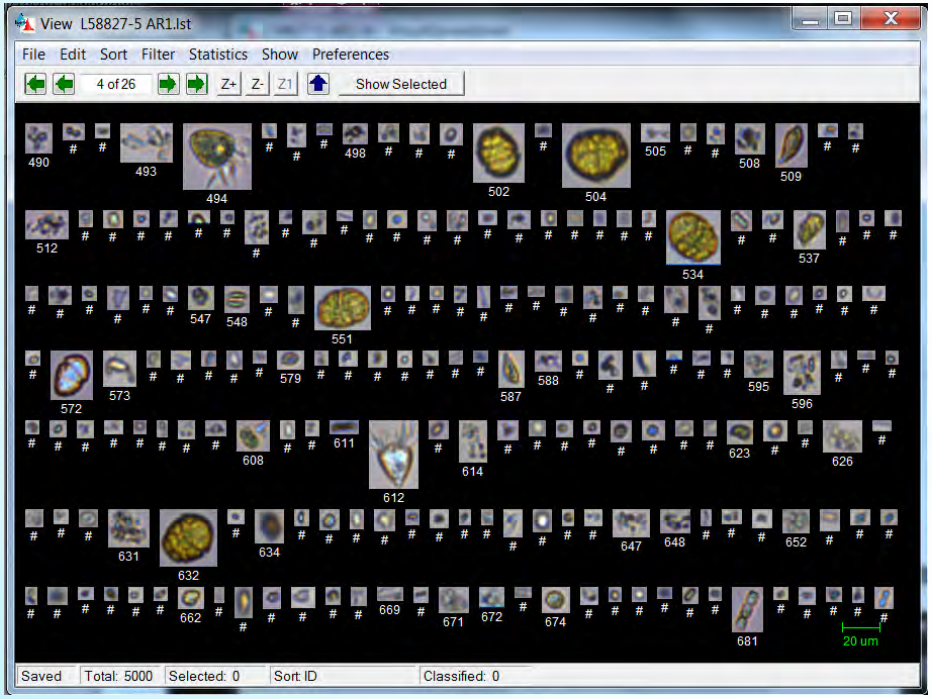
Magnification	Flow cell depth
4x	300 µm
10x	100 µm
20x	50 µm

Run time: 10-60 minutes

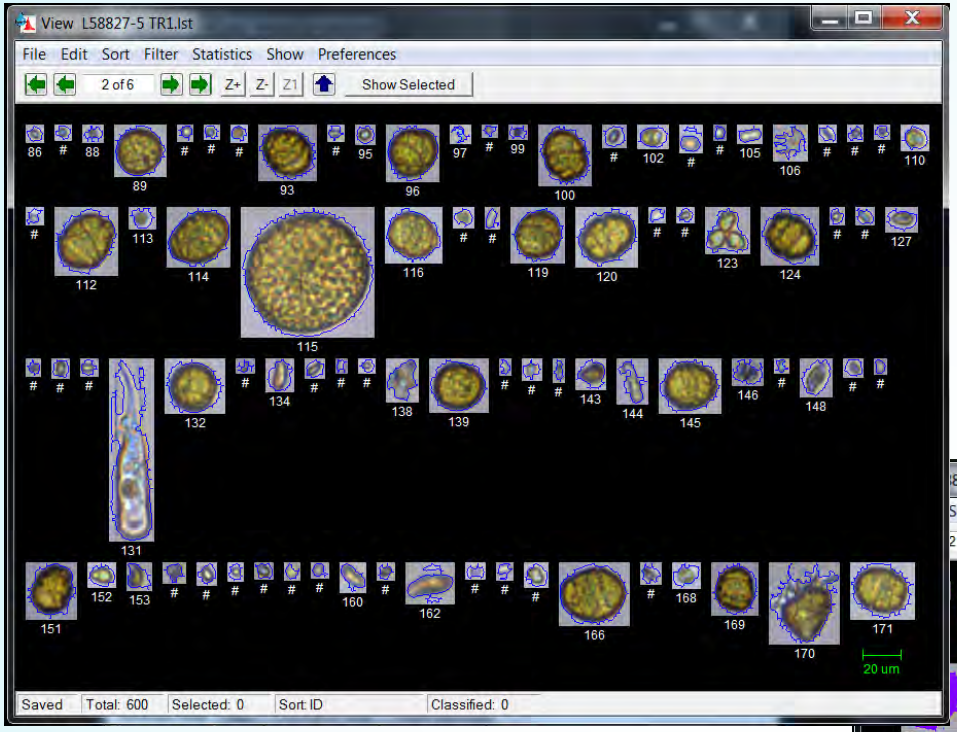
Yield: 1000-5000 images

Data Acquisition Modes

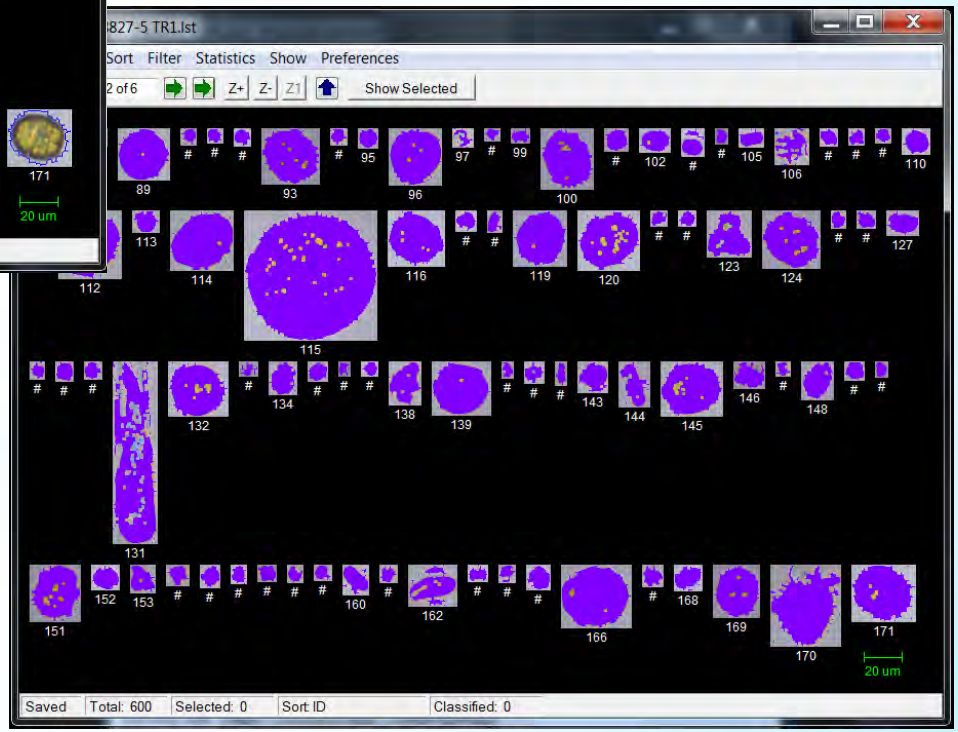
- **Autoimage Mode**: Captures particle images at a user-defined rate; images everything in field of view.
- **Trigger Mode**: Captures particle images only if a fluorescence signal is detected; images only chlorophyll containing cells.



Particle or not?



Binarization:
Pixels that are considered to be part of the particle are shown in purple.



Edge Trace:
The perceived edge of the particle is shown in blue.

Image Data Processing

- **Sort images using simple data filters**
  - e.g. size, shape, color

- **Sort images using complex pattern recognition**

  Goal: Auto-sorting into taxa by image recognition

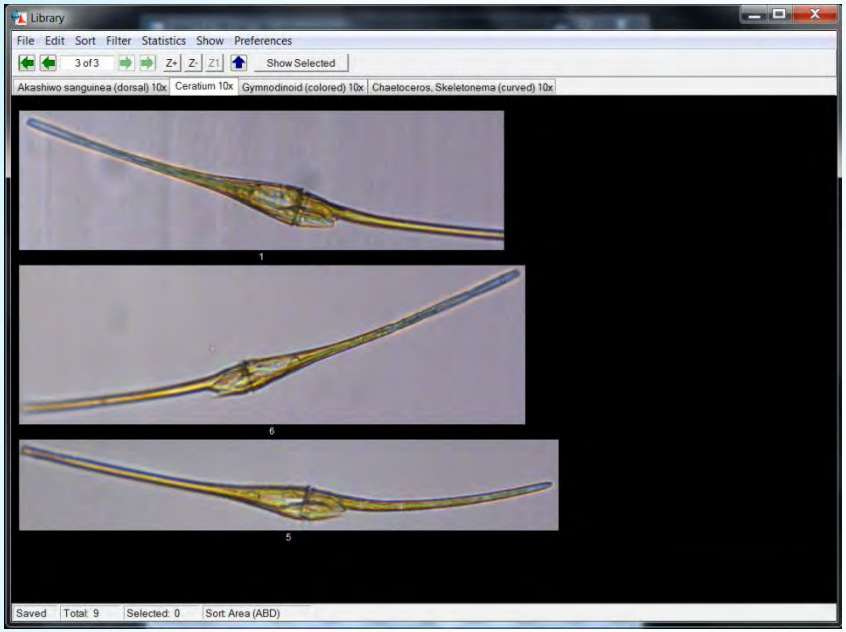
  - Build image libraries (taxa-specific)

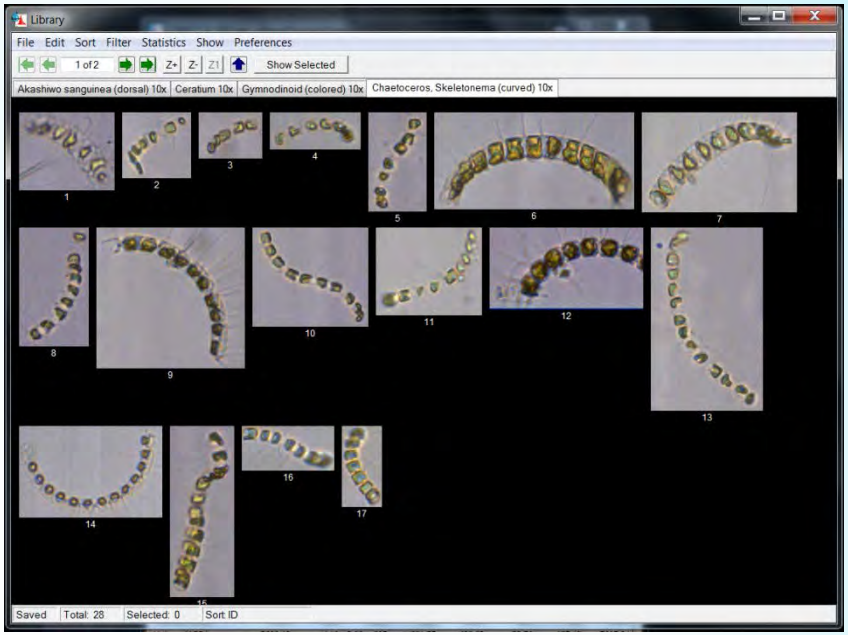
    → 'training sets' for pattern recognition
  - Used in advanced filter development
  - Based on combinations of large number of measured particle properties
  - Continuous development, limited capability, too many taxa…

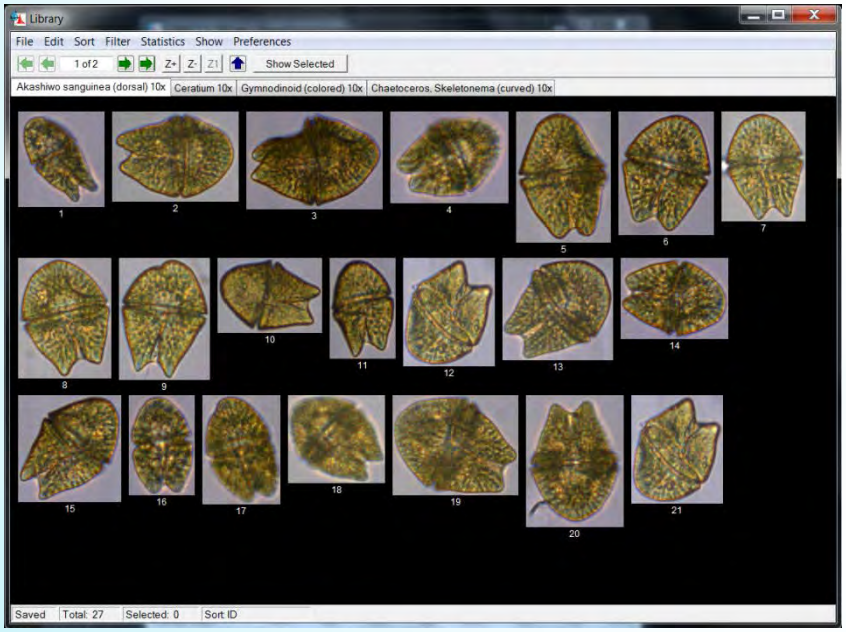
Particle Properties

measured and stored for each captured image

- ID #
- Area (ABD)
- Area (Filled)
- **Aspect Ratio**
- Average blue
- Average green
- Average red
- Calibration factor
- Calibration image
- Camera
- Capture X
- Capture Y
- Ch1 area
- Ch1 peak
- Ch1 width
- Ch2 area
- Ch2 peak
- Ch2 width
- Ch2/Ch1 ratio
- Circle fit
- **Circularity**
- Circularity (Hu)
- Compactness
- Convex perimeter
- Convexity
- D[4,3](ABD)
- D[4,3](ESD)
- Date
- **Diameter (ABD)**
- Diameter (ESD)
- Edge Gradient
- Elongation
- Feret angle max
- Feret angle min
- Fiber curl
- Fiber straightness
- Filter score
- Geodesic length
- Geodesic aspect ratio
- Geodesic thickness
- Image file
- Image height
- Image width
- Image X
- Image Y
- Intensity
- **Length**
- Particles per chain
- Perimeter
- Ratio blue/green
- Ratio red/blue
- **Ratio red/green**
- Roughness
- Scatter area
- Scatter peak
- Scatter width
- Sigma intensity
- Source image
- Sum intensity
- Symmetry
- Time
- Timestamp
- Transparency
- **Volume (ABD)**
- Volume (ESD)
- Width


Library
File Edit Sort Filter Statistics Show Preferences
3 of 3
Z+ Z- Z1
Show Selected
Akashiwo sanguinea (dorsal) 10x
Ceratium 10x
Gymnodinoid (colored) 10x
Chaetoceros, Skeletonema (curved) 10x
1
6
5
Saved
Total: 9
Selected: 0
Sort: Area (ABD)


Library
File Edit Sort Filter Statistics Show Preferences
1 of 2
Z+ Z- Z1
Show Selected
Akashiwo sanguinea (dorsal) 10x
Ceratium 10x
Gymnodinoid (colored) 10x
Chaetoceros, Skeletonema (curved) 10x
1
2
3
4
5
6
7
8
9
10
11
12
13
14
16
17
Saved
Total: 28
Selected: 0
Sort ID


Library
File Edit Sort Filter Statistics Show Preferences
1 of 2
Z+ Z- Z1
Show Selected
Akashiwo sanguinea (dorsal) 10x
Ceratium 10x
Gymnodinoid (colored) 10x
Chaetoceros, Skeletonema (curved) 10x
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
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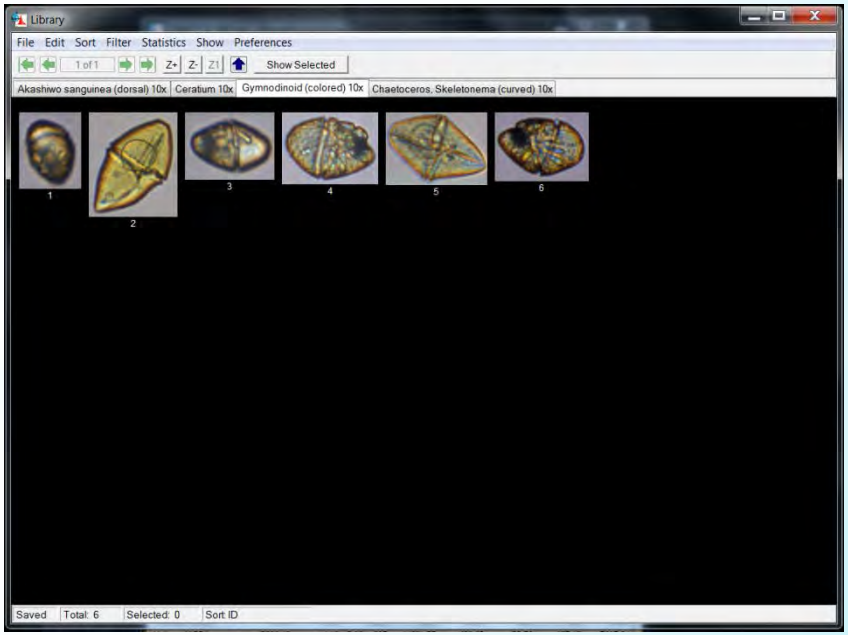
Sort ID


Library
File Edit Sort Filter Statistics Show Preferences
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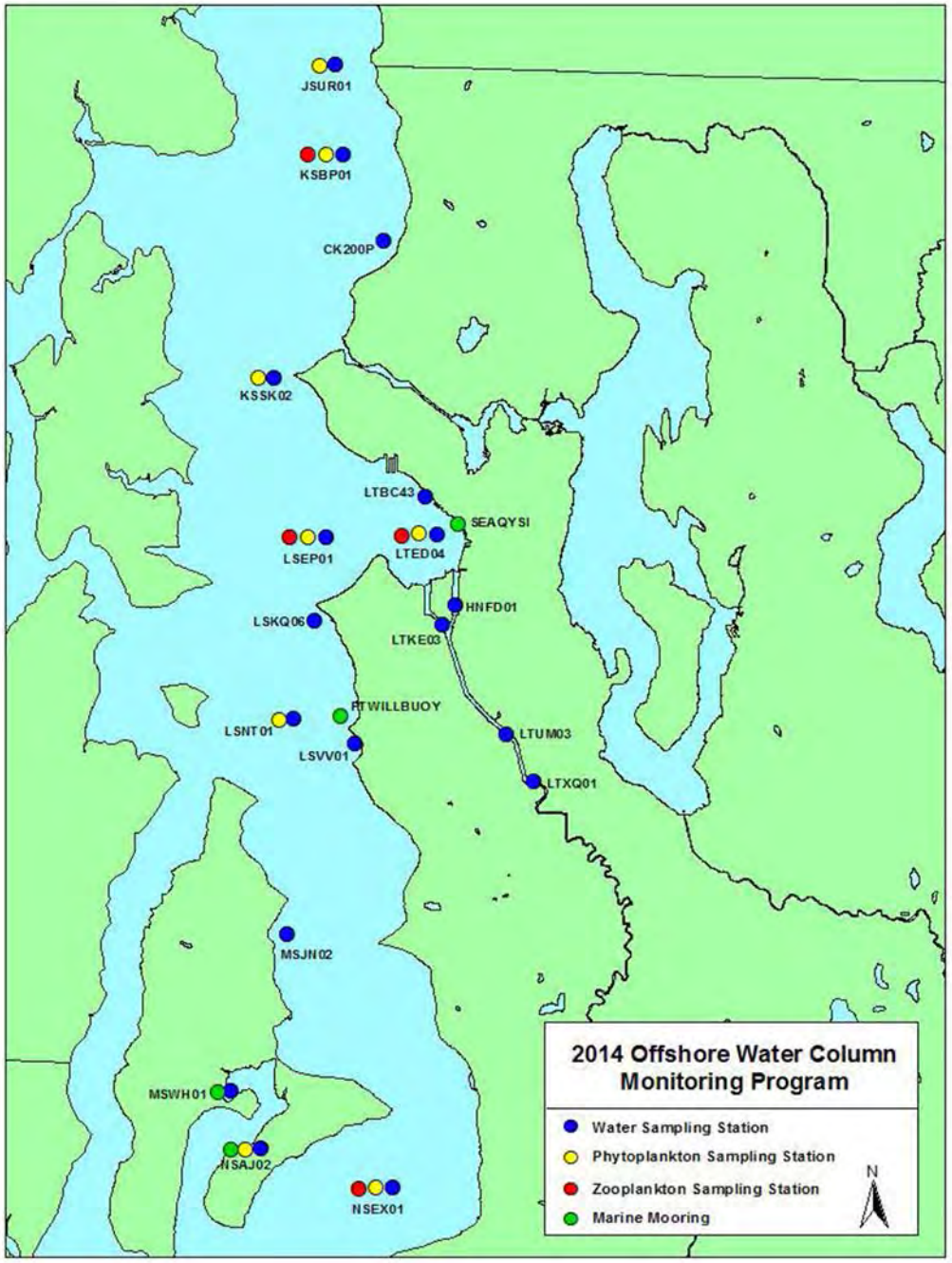
Z+ Z- Z1
Show Selected
Akashiwo sanguinea (dorsal) 10x
Ceratium 10x
Gymnodinoid (colored) 10x
Chaetoceros, Skeletonema (curved) 10x
1
2
3
4
5
6
Saved
Total: 6
Selected: 0
Sort ID

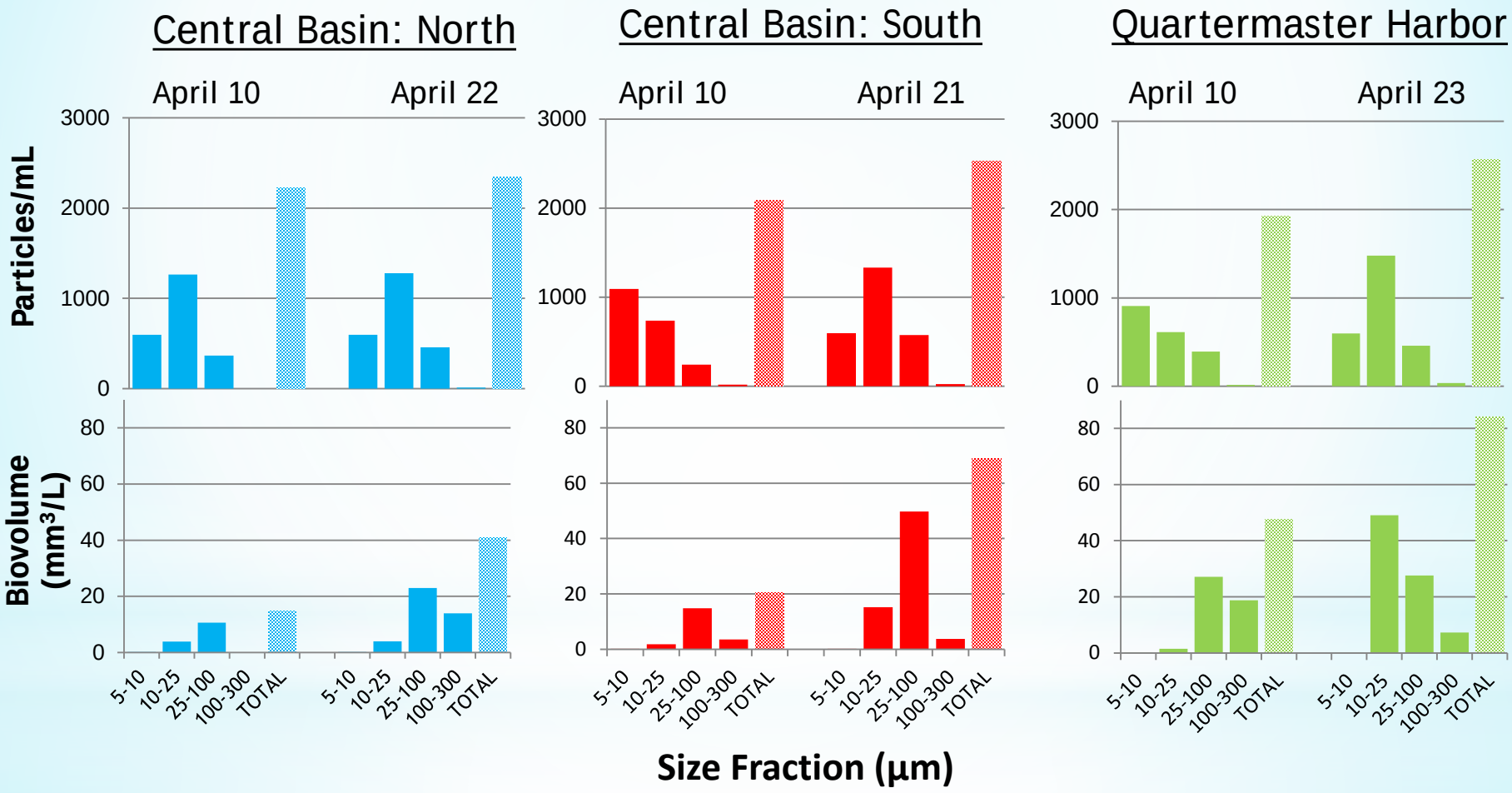
2014 Sampling

Pt. Jefferson
East Passage
Dockton Pk.

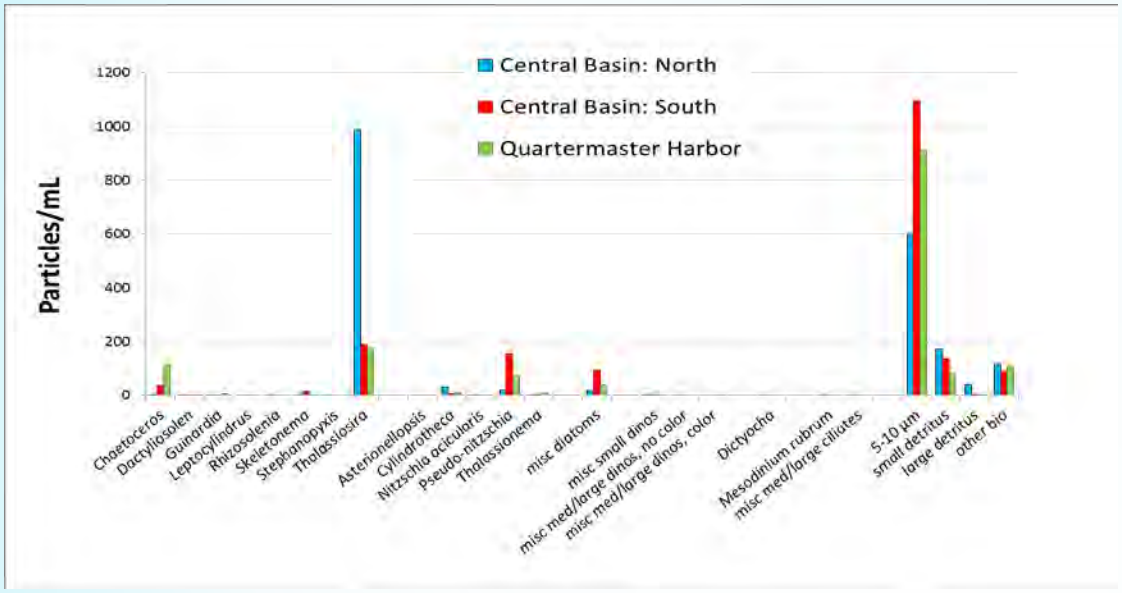
Point Wells
Westpoint TP Outfall
Elliott Bay
South TP Outfall
Fauntleroy

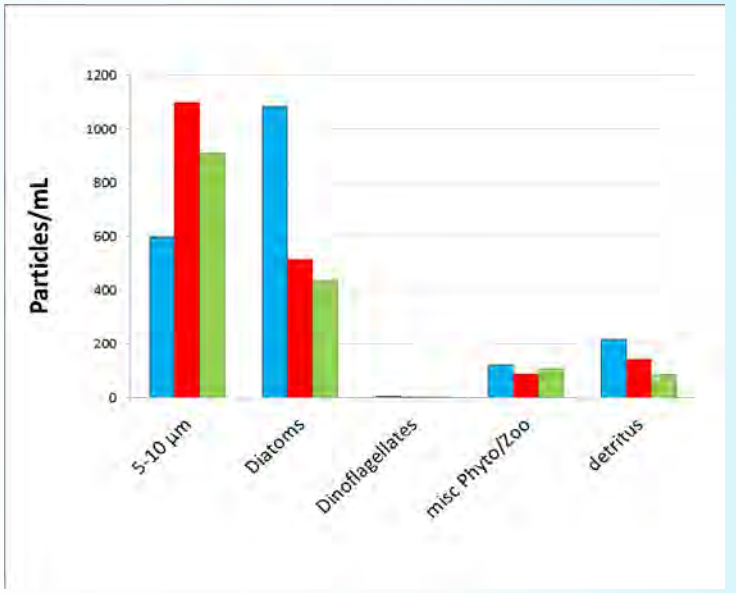


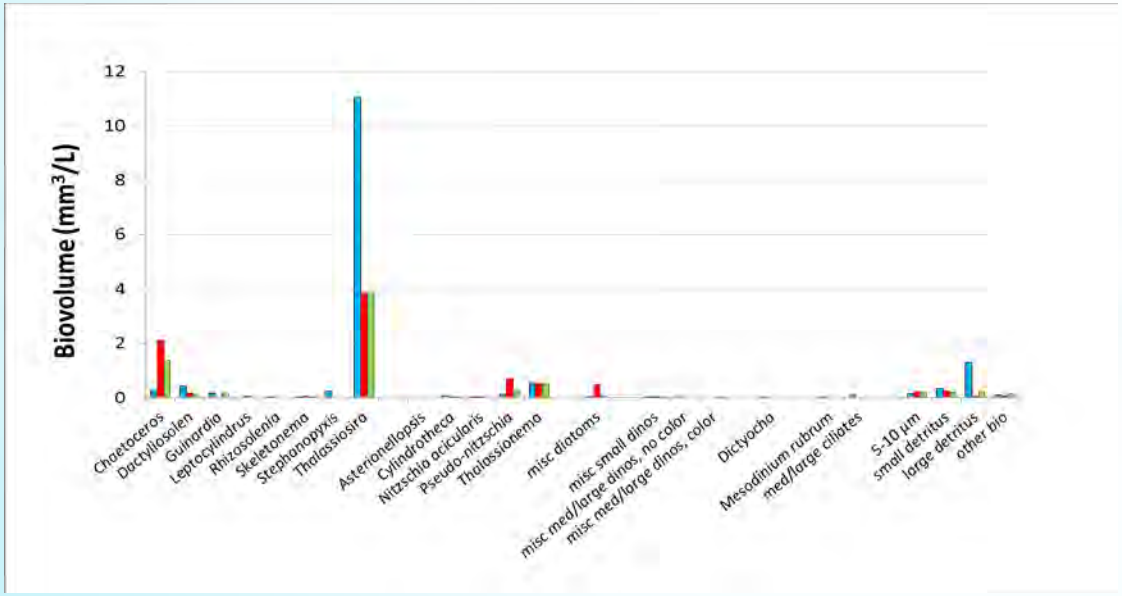
2014 FlowCAM Data

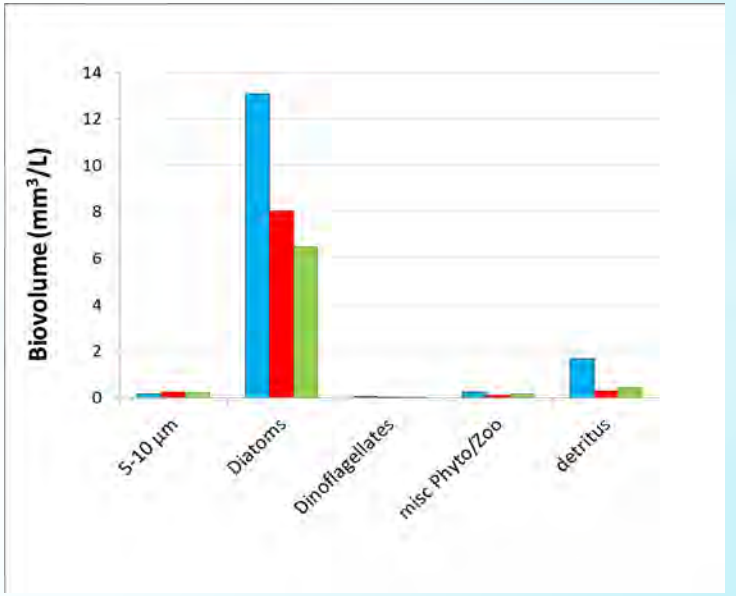


April 10, 2014
Taxa classification using FlowCAM
(<100 µm fraction)



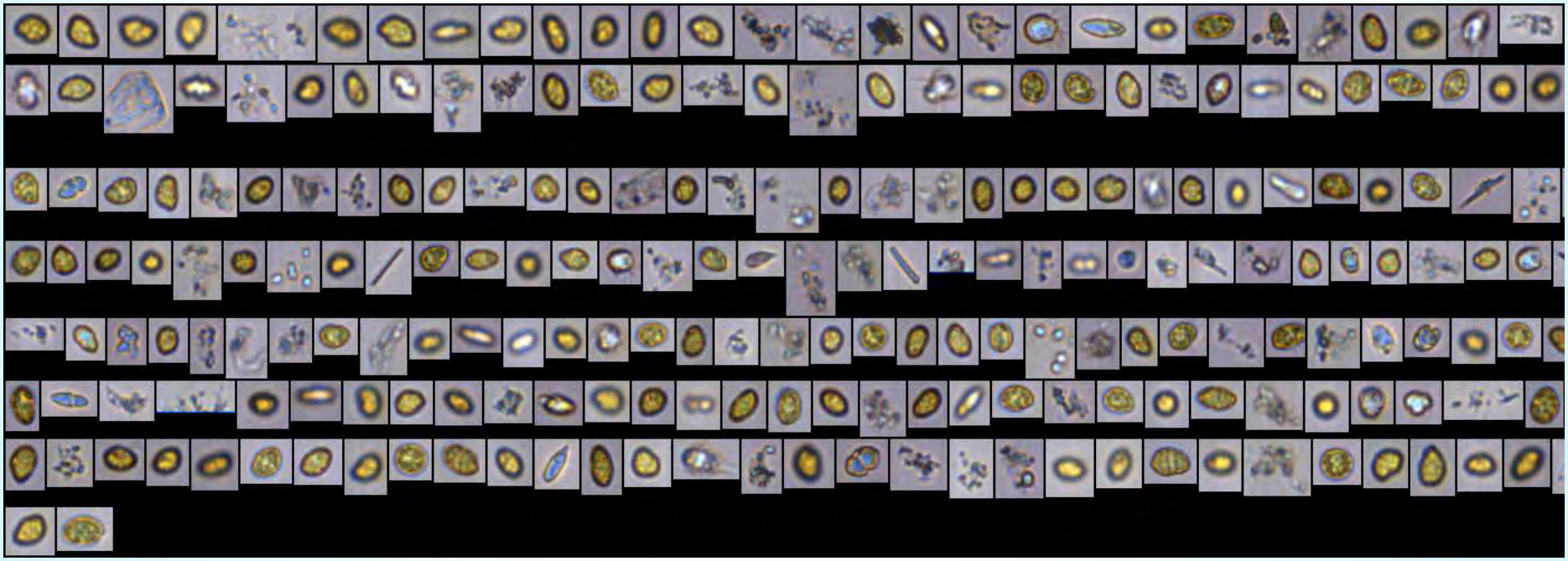






Analysis of Harmful Algal Blooms

July 2013: *Heterosigma* bloom implicated in Quartermaster Harbor fish kill



12-17 µm ABD (area based diameter) size fraction

Technical Challenges

- **Flow cell size limit**- currently using 100 µm and 300 µm
  - Exchanging objectives takes time - Not practical for each sample
  - Magnification-specific libraries are needed for image recognition
- Image resolution
- Long run times for sparse samples (>60min)
- Image libraries have significant limitations (natural heterogeneity)
- Taxa sorting is limited by capability of the software

  → Lots of manual sifting and sorting
- Data management
  - Data volume, processing speed of large files, primitive output

Acknowledgments

Special thanks to the

King County Environmental Lab Field Unit