Field Demonstration of a Novel Biofouling Control Technology for Micro-Channel Flow Cells

Carlton D. Hunt[1], Derek Michelin[1], Henry Pate[3], Brian Sikorski[2], Michael Neal[2], Caleb Chitwood[2], Harry Nelson[4]

- 1Battelle, 397 Washington Street, Duxbury, MA
- 2Battelle, 505 King Avenue, Columbus, OH
- 3Battelle Florida Materials Research Facility, Ponce Inlet, FL
- 4Fluid Imaging Technologies, Inc., Yarmouth, ME

Environmental Measurement Symposium 2012
Washington, DC

Overview

- Technology need
- Introduction the Submersible FlowCAM® (an *in situ* particle/plankton sensor)
- Overcoming biofouling for long term deployments
- Testing and Deployments
- 45 day time series, Ponce Inlet, FL
- Conclusions

Why a submersible particle imaging tool?

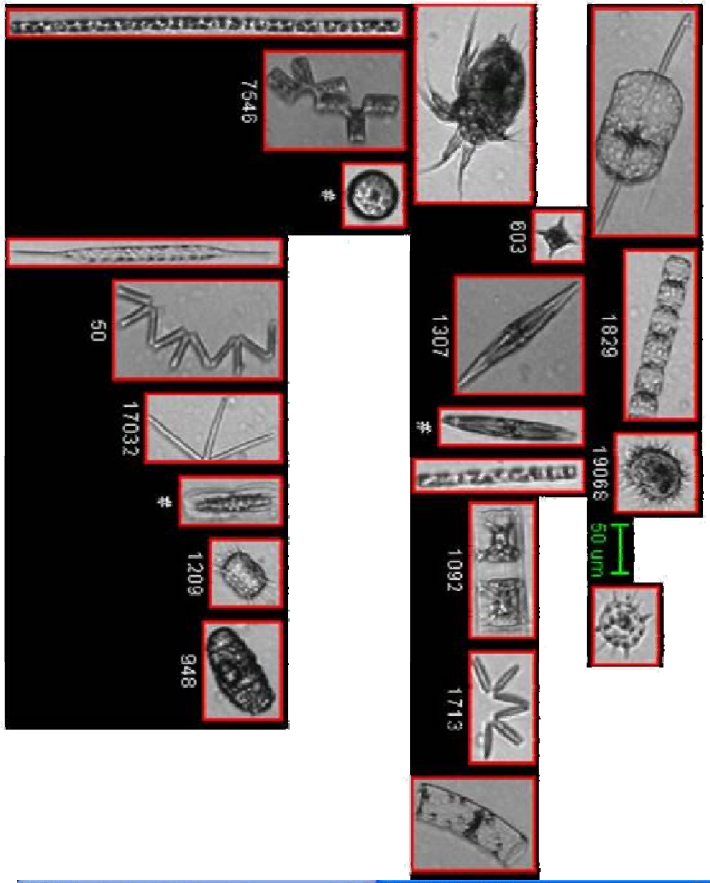
- **Application Needs**
  - Harmful Algal Bloom (HAB) monitoring (e.g., red tides)
  - Noxious algal monitoring in drinking water reservoirs
  - Health and safety of aquaculture operations
  - Sewage effluent discharge monitoring
  - Carbon Cycling in the coastal and deep ocean
  - Ocean Observing System (IOOS) moorings
- **Traditional measurement tools**
  - Collect discrete sample (frequency and depth)
  - Field Preserve / Concentrate in laboratory settling
  - Taxonomist manually counts species and abundance
  - Limits timeliness of data and spatial/temporal understanding/response
- **Bench /portable particle imaging instruments**
  - Gaining ground in environmental studies, including oil particle characterization (e.g., FlowCAM®)

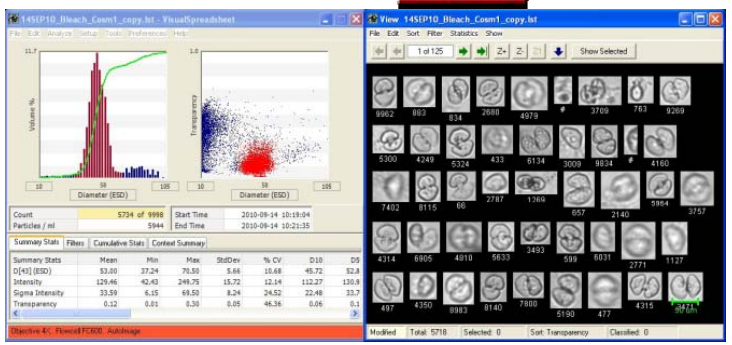
Underwater Instrumentation Challenges

- **Reliable operation in a wet, high pressure, corrosive environments.**
- **Biofouling management and control during long-term deployments.**
- **Our solution**
  - Engineer a commercial instrument (a portable FlowCAM®) for underwater operations.

FlowCAM® – Features

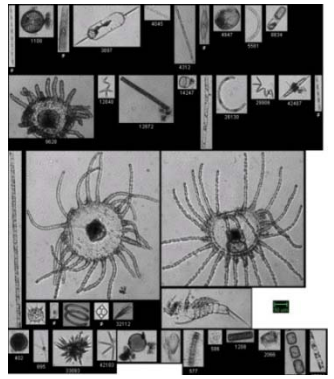
- **Based on flow cytometry concepts**
- **Captures images of all particles in field of view**
  - Grayscale or color
- **Particle size range (3µm – 3mm)**
- **Multiple Image Acquisition Methods**
  - Two Channel Fluorescence detection
  - Auto (fixed frame rate of 1-22 frames/sec)
  - Forward scatter
- **VisualSpreadsheet©**
  - Measures and records 30 parameters per particle imaged
  - Image Recognition with Automated Identification, Classification & Enumeration





Submersible FlowCAM® Development

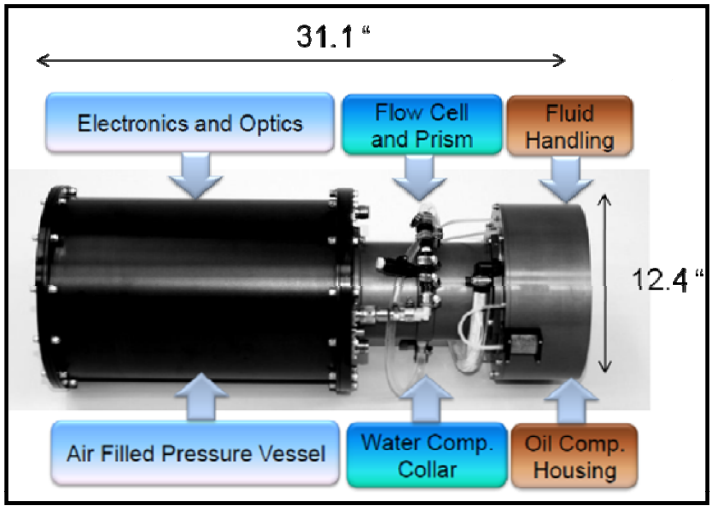
- Battelle and Fluid Imaging Technologies (FIT) teamed to develop a submersible FlowCAM® (SFC)
  - Enables real time, in situ particle imaging
- Features
  - **200 meter depth rating**
  - **1X, 4X, or 10X magnification**
  - **Autonomous operation**
    - Programmable on/off cycles
    - Configurable triggering (auto, scatter, fluorescence)
  - **Flexible deployment**
    - Buoy, Pier, ROV, AUV, Shipboard or Fixed-Platform Profiling, Bench
  - **Remote configuration, system checking, and control**



SFC System Overview

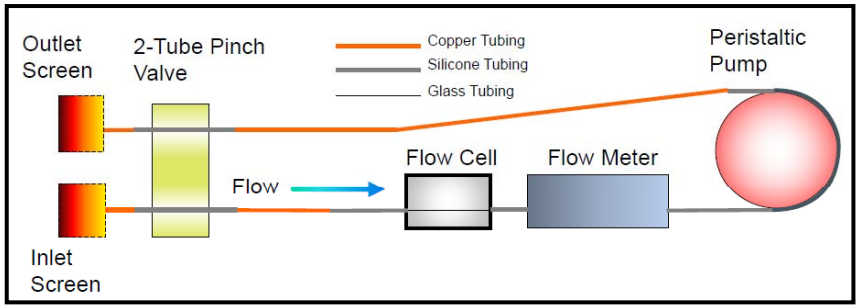
System Layout

- **Three joined housings**
  - **Air filled:** electronics & optics (1 atm)
  - **Water compensated:** sampling chamber
  - **Oil filled:** contains fluid delivery pump



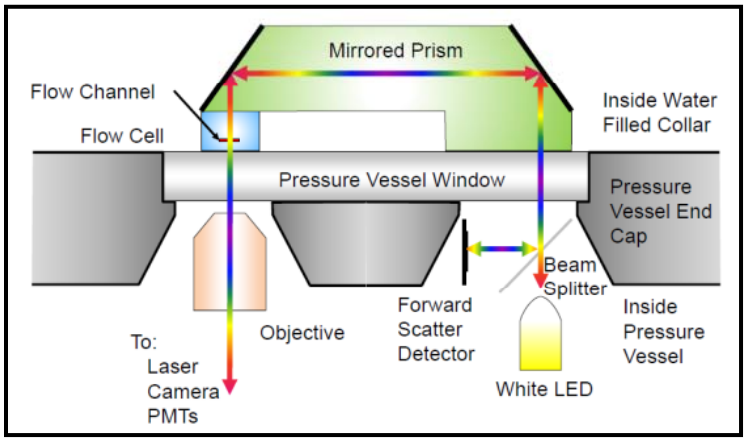
Fluid Flow Path

- Entirely pressure compensated
- Peristaltic pump
- 1/16” ID Tubing (**low cost, removable**)
- Glass capillary flow cell

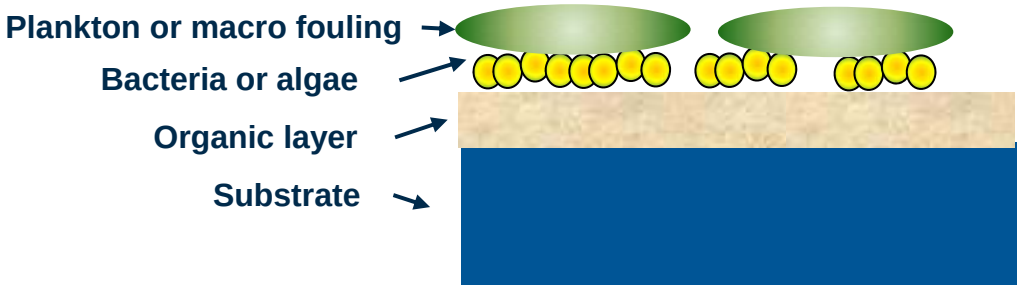


Optical Path

- Imaging done across **pressure boundary**
- Unique **mirrored prism** ensures optical path is clean (patent pending)



Biofouling Control Goals & Approach

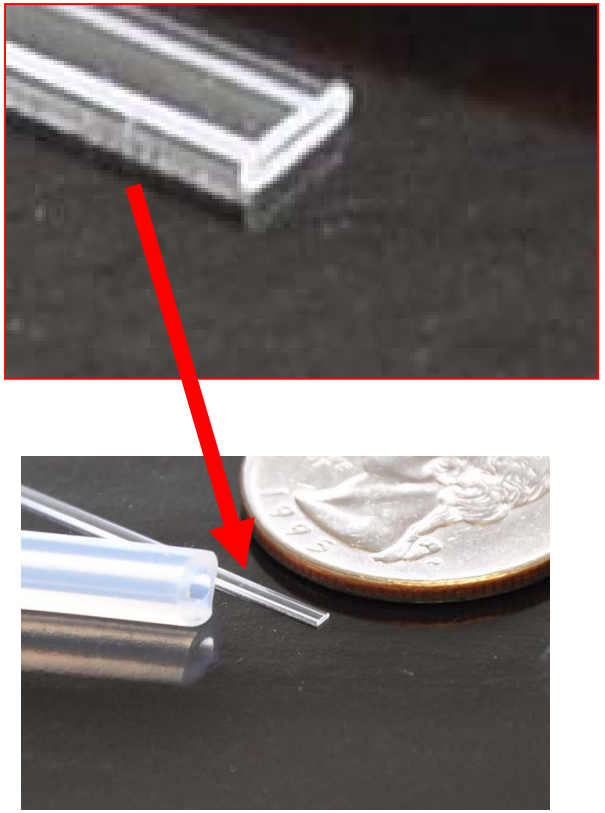


Challenge

- **Ensure flow** through the flow cytometry cell and system
- Maintain **clean optical surfaces** to facilitate high quality imaging

Approach

- Two independent systems
  - **Copper:** Included in initial design
  - **UV:** Evaluated in parallel to system development



Biofouling Control Method

- **Copper**
  - (A) Cu dissolves and damage cell membrane
  - (B) Cell membrane ruptures
  - (C) Cu ions cause oxidative reactions within the cell
  - (D) Genomic and plasmid DNA degrades
- **UV**
  - Impedes adsorption of organic molecules on glass surface
  - Breaks molecular bonds in organic molecules like DNA
  - Either inhibits reproduction or kills cells

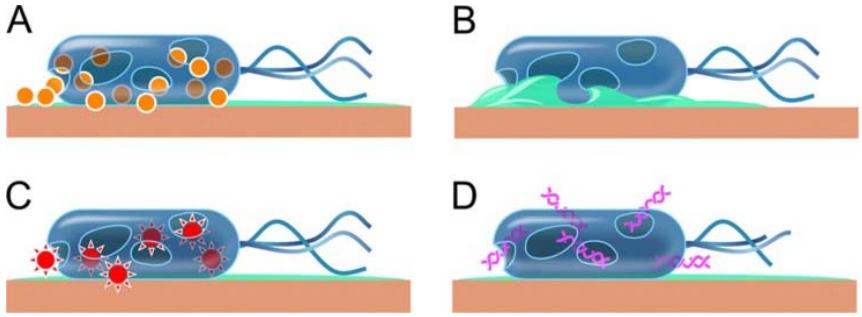


Image: Applied Environmental Microbiology

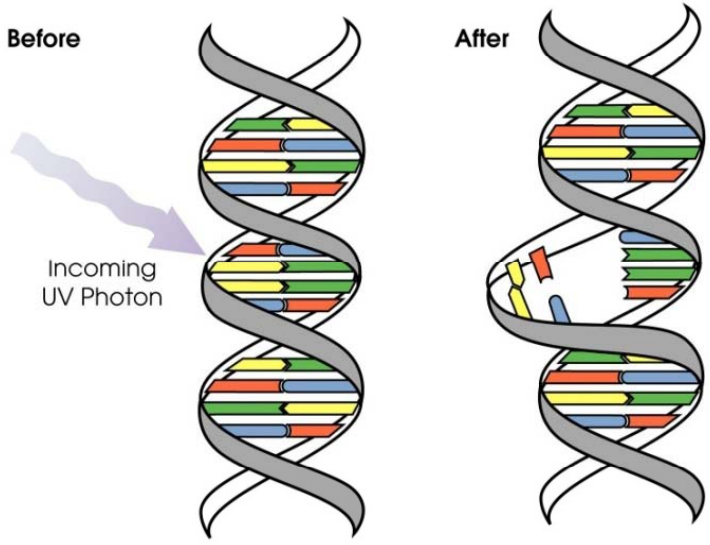
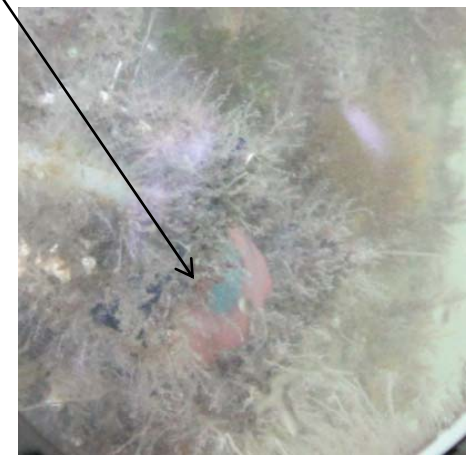


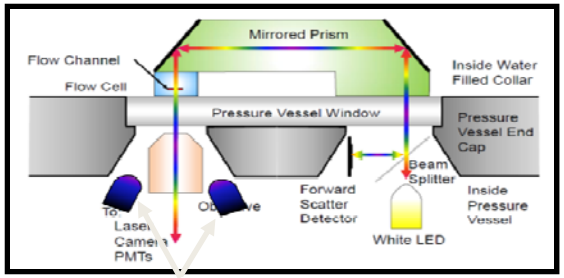
Image: NASA

Biofouling Control System Implementation

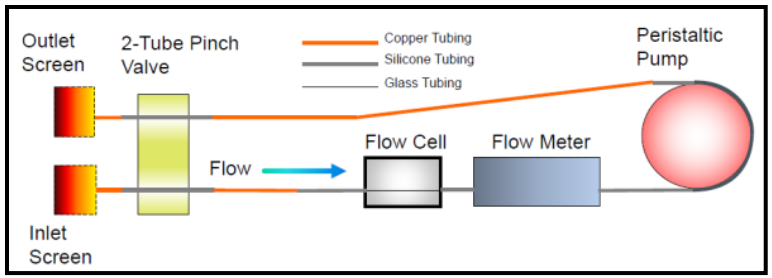
- **Copper**
  - Use **copper mesh** at inlet/outlet
    - Inhibits growth
    - Prevents ingestion of large particles that may cause mechanical fouling
  - Use **copper tubing** in flow path
    - 12” section before and after pump
- **UV LEDs illuminate flow cell**
  - Inhibit growth on glass



50 days under water

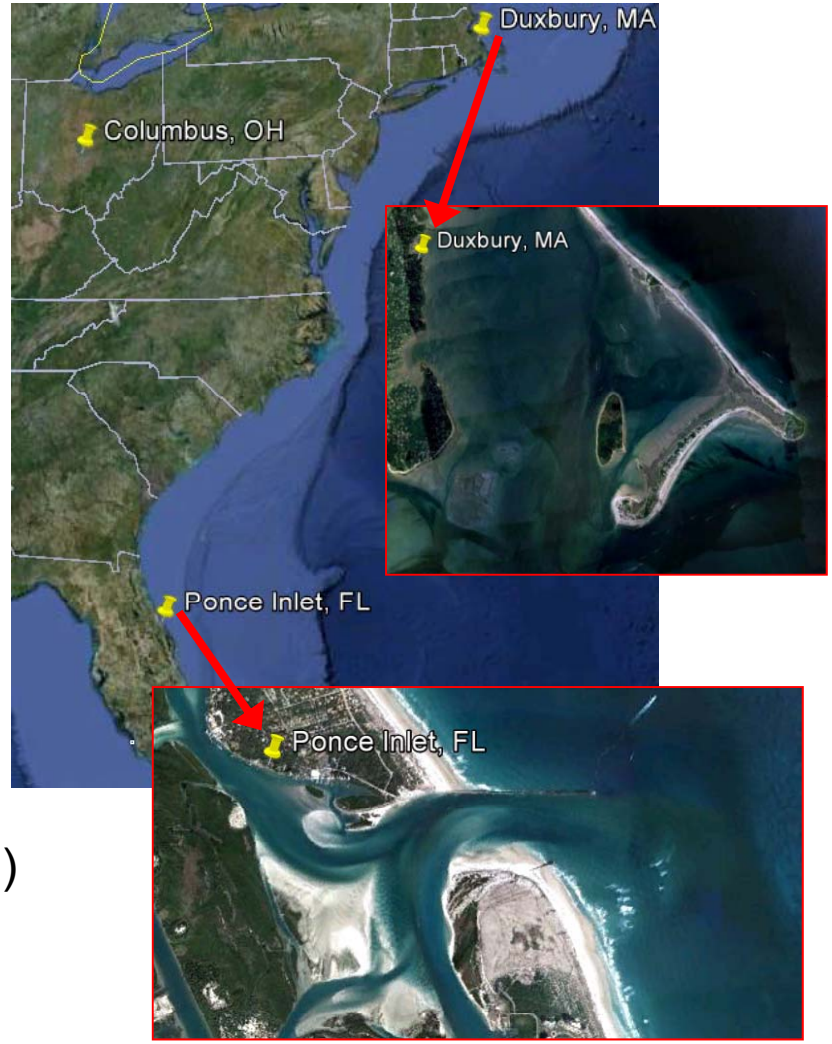


UV LEDs



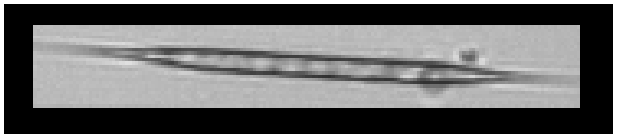
Testing Overview

- **Initial Sea Trial Profiling (Cu only)**
  - System test Duxbury, MA (Feb 2010)
- **Biofouling Control Concept Tests**
  - Lab test Ponce Inlet, FL (Jan – Feb 2010)
- **UV Exposure Testing**
  - Lab Test Ponce Inlet, FL (Aug – Sep 2010)
- **Lab Endurance Test (copper only)**
  - System test Duxbury, MA (Sep 2010)
- **Field Endurance Test (Copper + UV)**
  - System test Ponce Inlet, FL (Jan – Mar 2012)

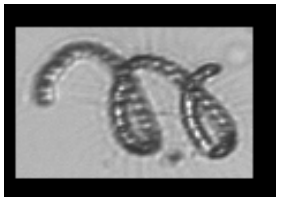


Test Results Summary
Initial Sea Trial (Feb 2010)

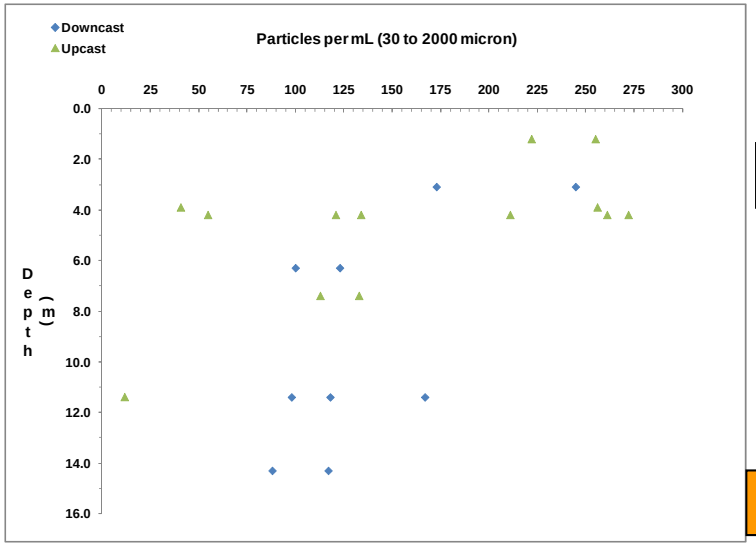
- **Approach**
  - Lowered system over side from R/V in Plymouth Bay, MA
  - Collected data at multiple depths during descent/ascent
  - Water temp, air temp approx 0 C°
- **Results**
  - System worked successfully to 14 m
  - Obtained excellent data in adverse conditions
  - No fouling issues

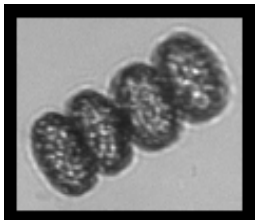


Rhizosolenia



Chaetoceros

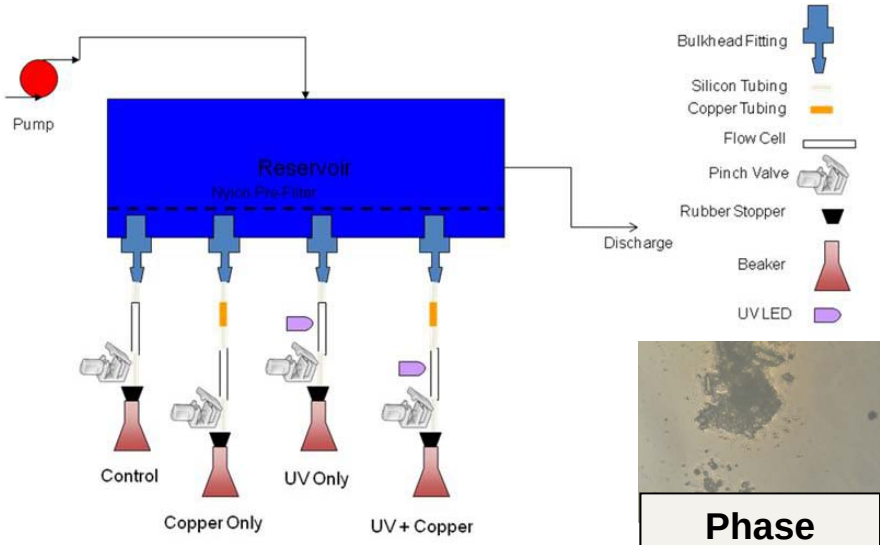




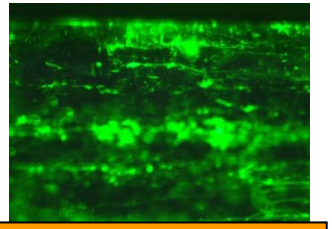
Thalassiosira

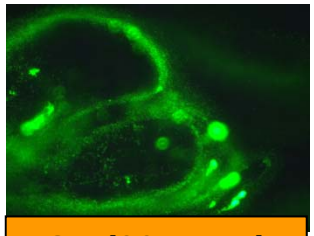
Test Results Summary
Biofouling Control Tests (Jan – Feb 2010)

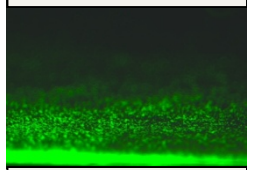
- **Approach**
  - Tested 3 samples of each method (Cu, UV, UV+Cu) for down-select
  - Up to **63 day exposure** of two flow cells with constant flow of filtered seawater
  - Samples analyzed at Battelle and FIT
- **Results**
  - **No Treatment (Control):** Heavy fouling, no flow within 48 days
  - **Cu:** Biofouling and flow reduction after 48 days
  - **UV:** Biofouling and small flow reduction after 48 days
  - **UV+Cu:** No flow reduction; minor biofouling after 63 days

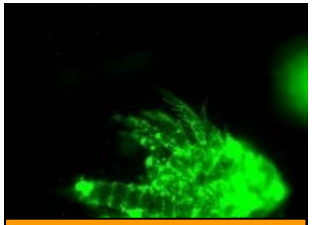


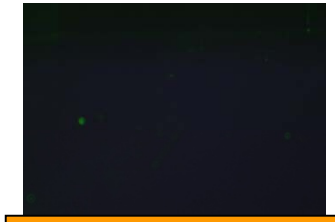
Phase Contrast

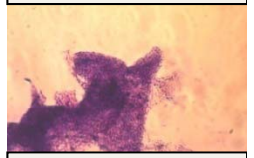

Control (60 Days)


Cu (60 Days)


Nucleic Acid Stain

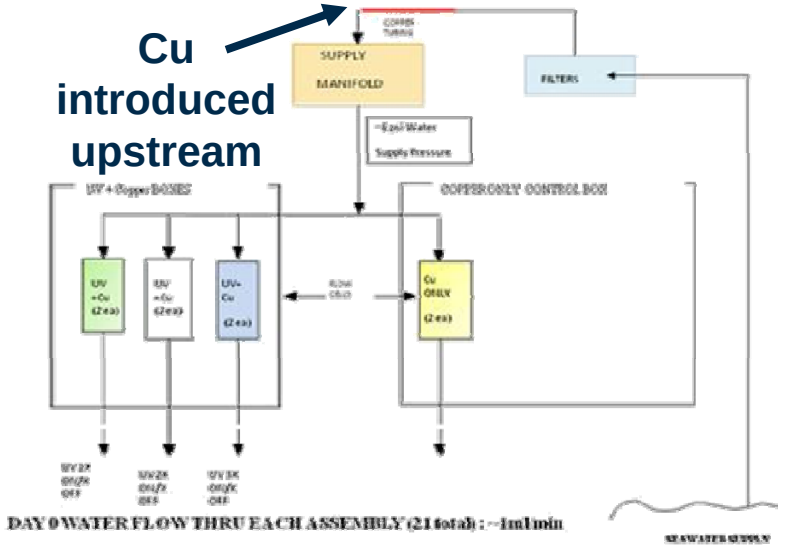

UV (49 Days)

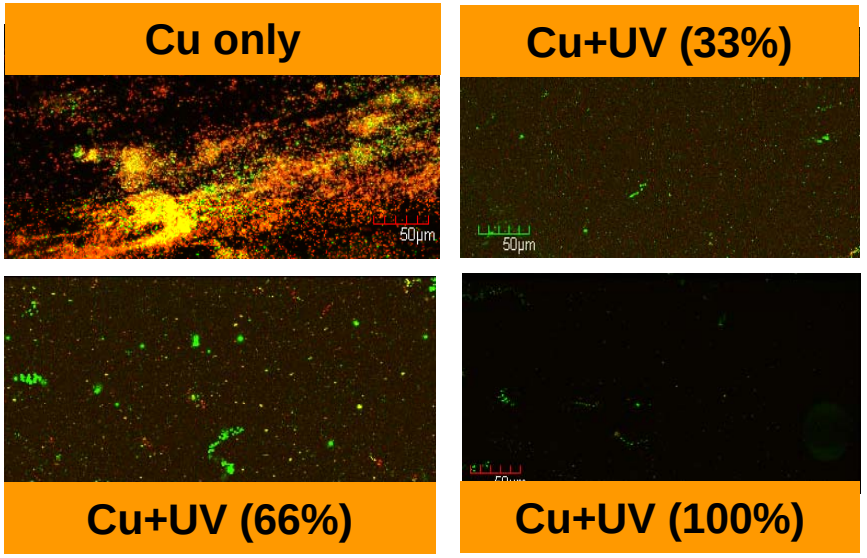

UV+Cu (60 Days)


Capsule Stain

Test Results Summary
UV Exposure Testing (Aug – Sep 2010

- **Approach**
  - Tested three cytometry tubes at three UV doses (33%, 66%, 100%) on Cu
  - **36 day exposure** of flow cells with constant flow of filtered seawater
  - Confocal microscope and staining used to image fouling in flow cells
- **Results**
  - Biofilm accumulation on Cu-only samples similar to previous result
  - Effectiveness of Cu+UV treatment similar to previous test results
  - Results similar for all UV dosage levels (33%, 66%, 100%)



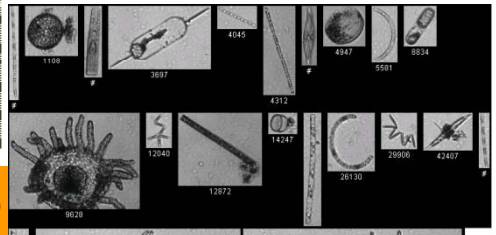


Field Endurance Test (Jan – Mar 2012)

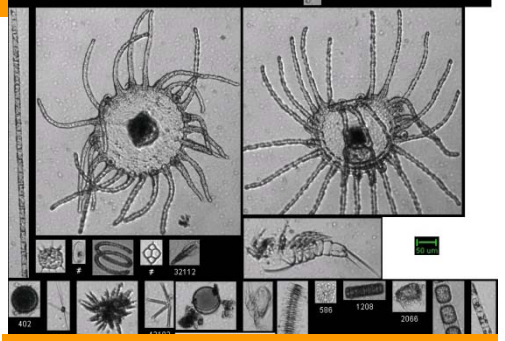
- **Assess biofouling and corrosion during 30+ day deployment**
  - Fully automated; remotely operated
  - Data acquisition for fifteen (15) minutes of every three (3) hours
  - UV always on
  - Depth: 3 to 7.5 ft; Temp: 15 to 22ºC
  - Triggering: Chlorophyll & scatter
  - Size threshold: 20 µm to 3000 µm
  - Background calibration at start of each run

Battelle Materials Research Center – Ponce Inlet, FL

External Fouling – Day 45



Calibration Image – Day 49



SFC Images – Day 43

SFC Setup

Date/Time:

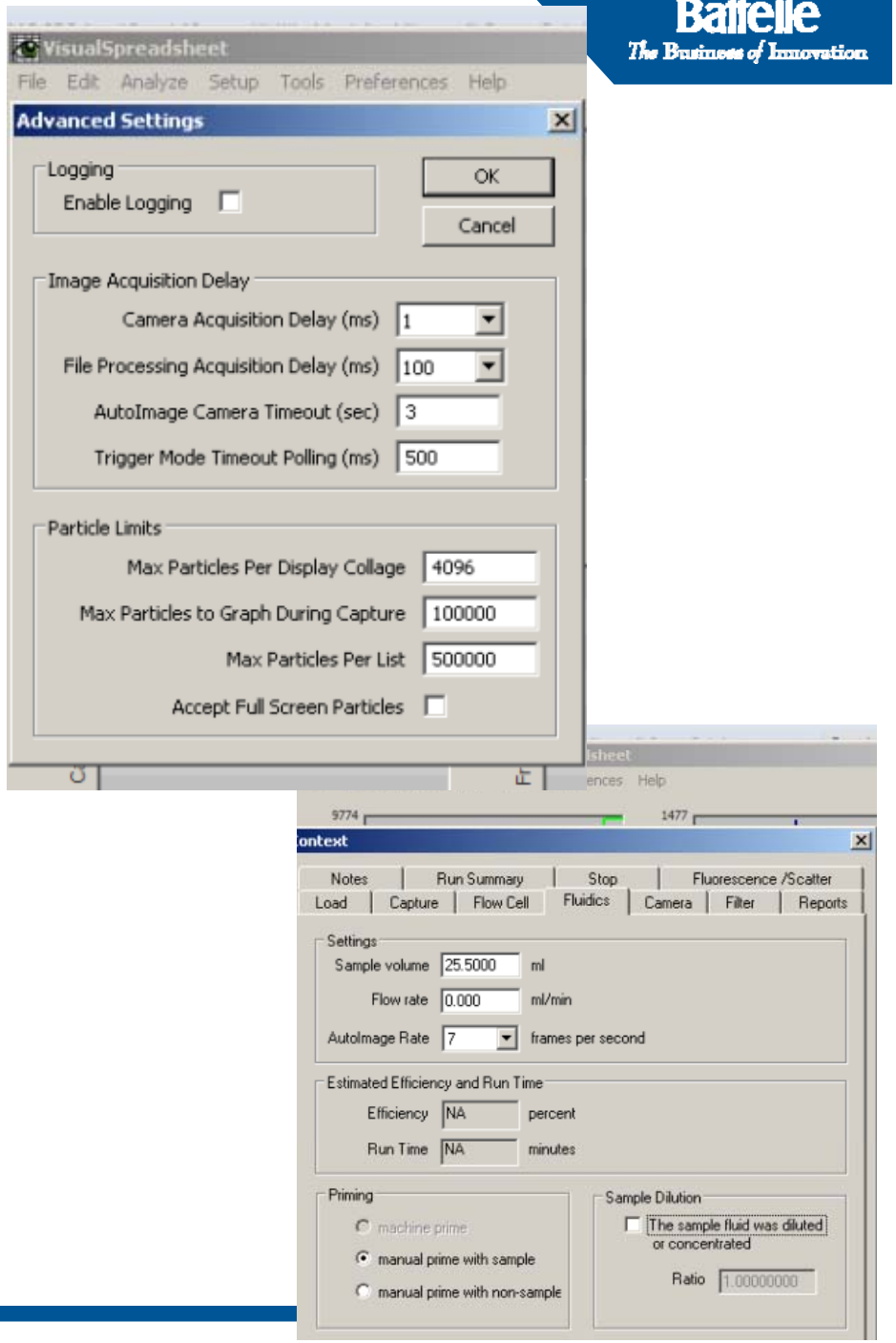
Start: 2012-02-02 11:08:19
End: 2012-02-02 11:23:10
Sampling Time: 00:14:44

Run:

Mode: Trigger
Recalibrations: 0
Stop Reason: User Terminated
Fluid Volume Imaged: 1.9103 ml
Particle Count: 9255

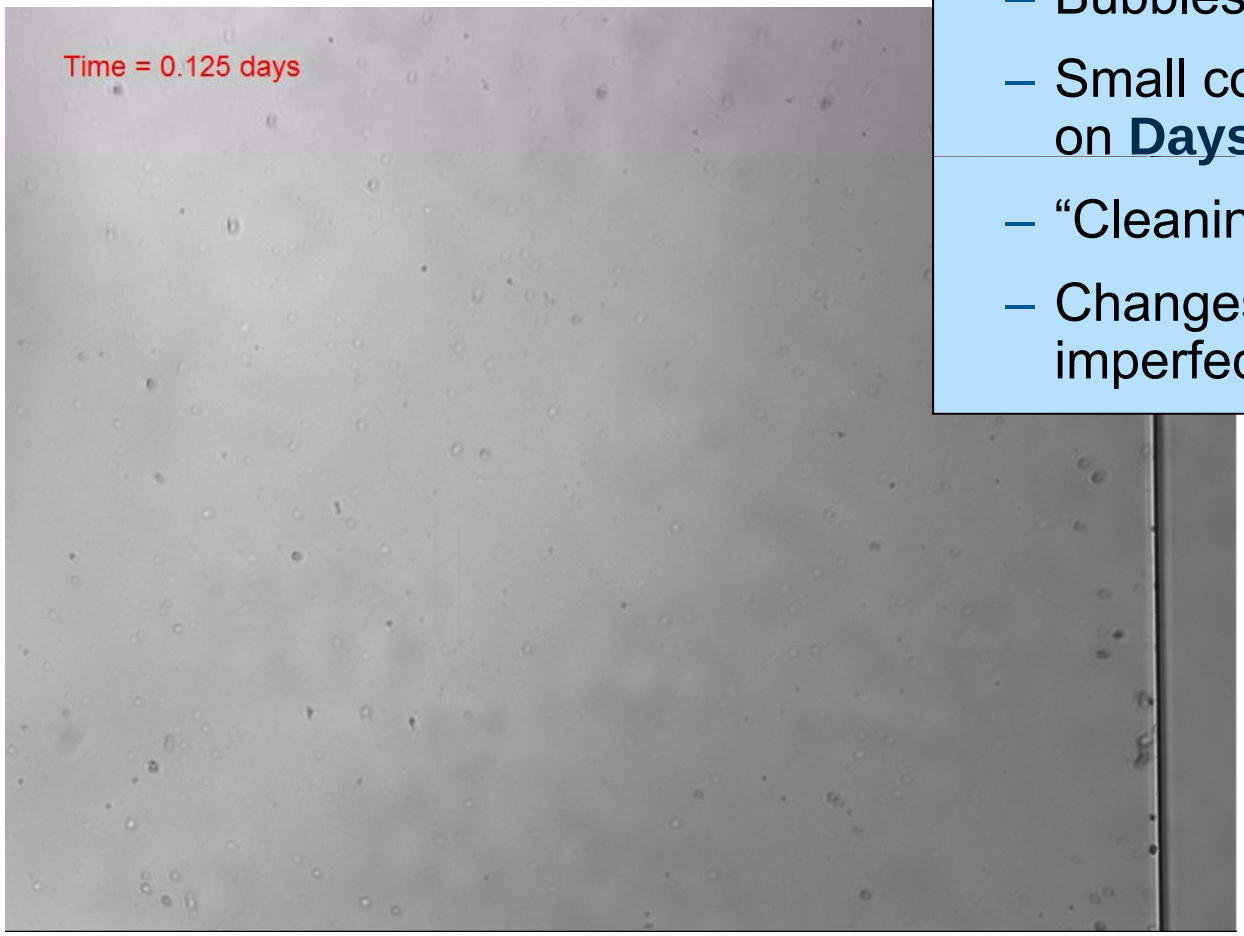
Images:

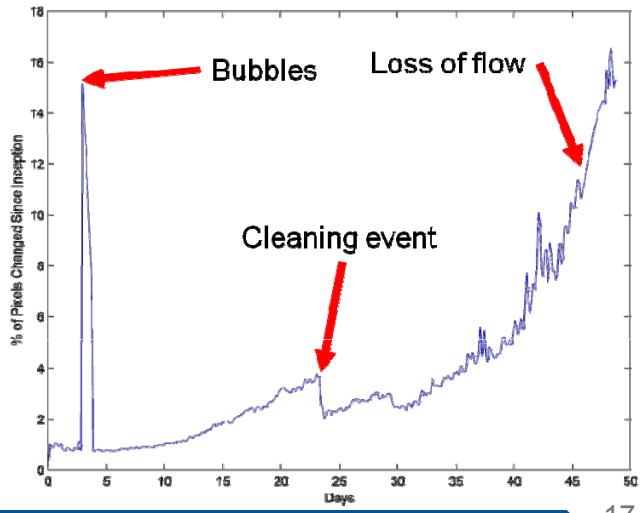
Total: 2583
Used: 1482
Percentage Used: 57.38%
Particles Per Used Image: 6.24
Frame Rate: 2.92 fps
Intensity Mean: 154.86
Intensity Min: 153.08
Intensity Max: 155.78



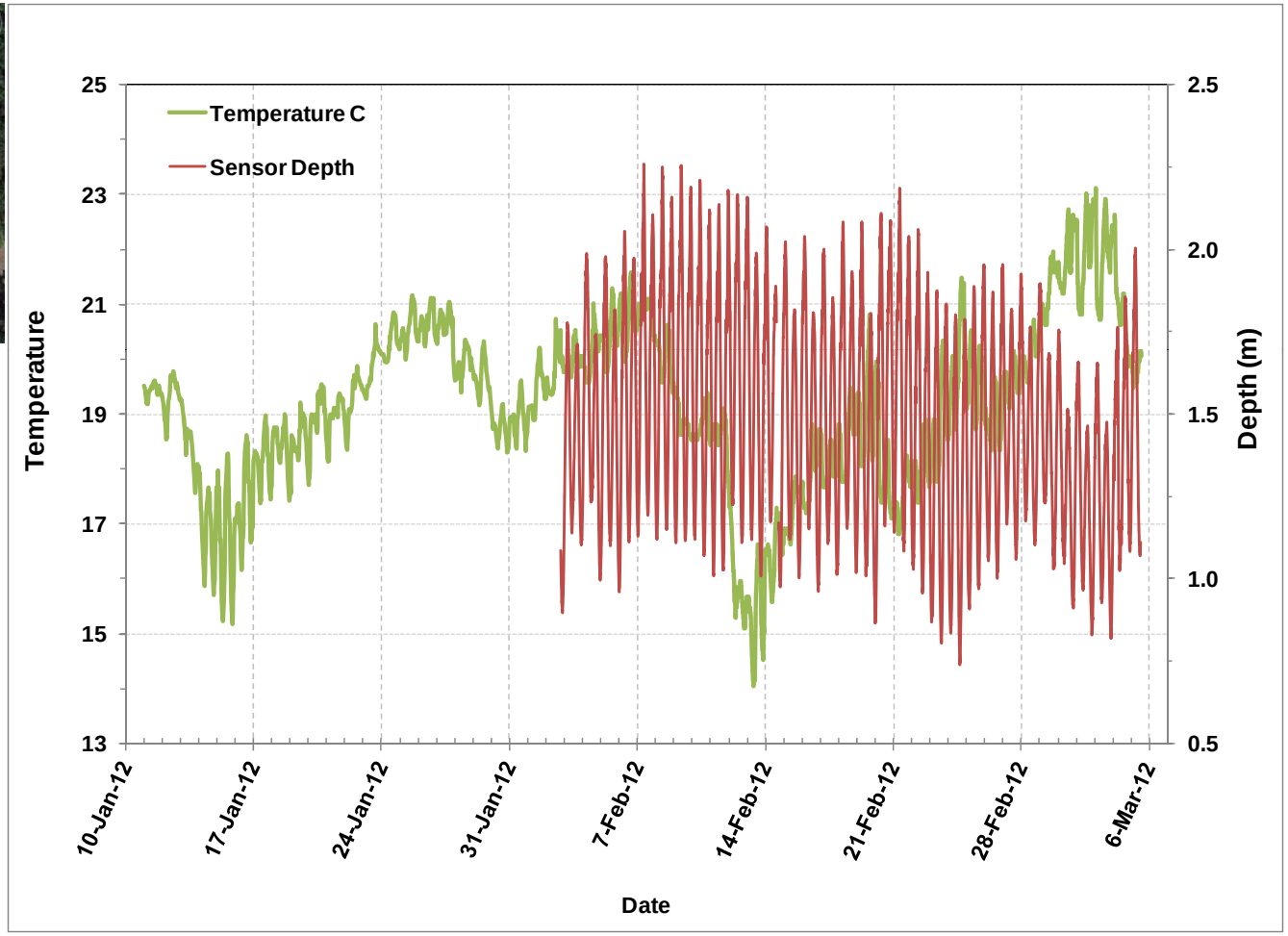
Video of Calibration Images
Field Endurance Test (Jan – Mar 2012)

- Features to look for:
  - Flow cell edge movement
  - Bubbles around **Day 3**
  - Small colonies being washed away on **Days 14 & 22**
  - "Cleaning event" on **Day 23**
  - Changes in lens/lighting imperfections

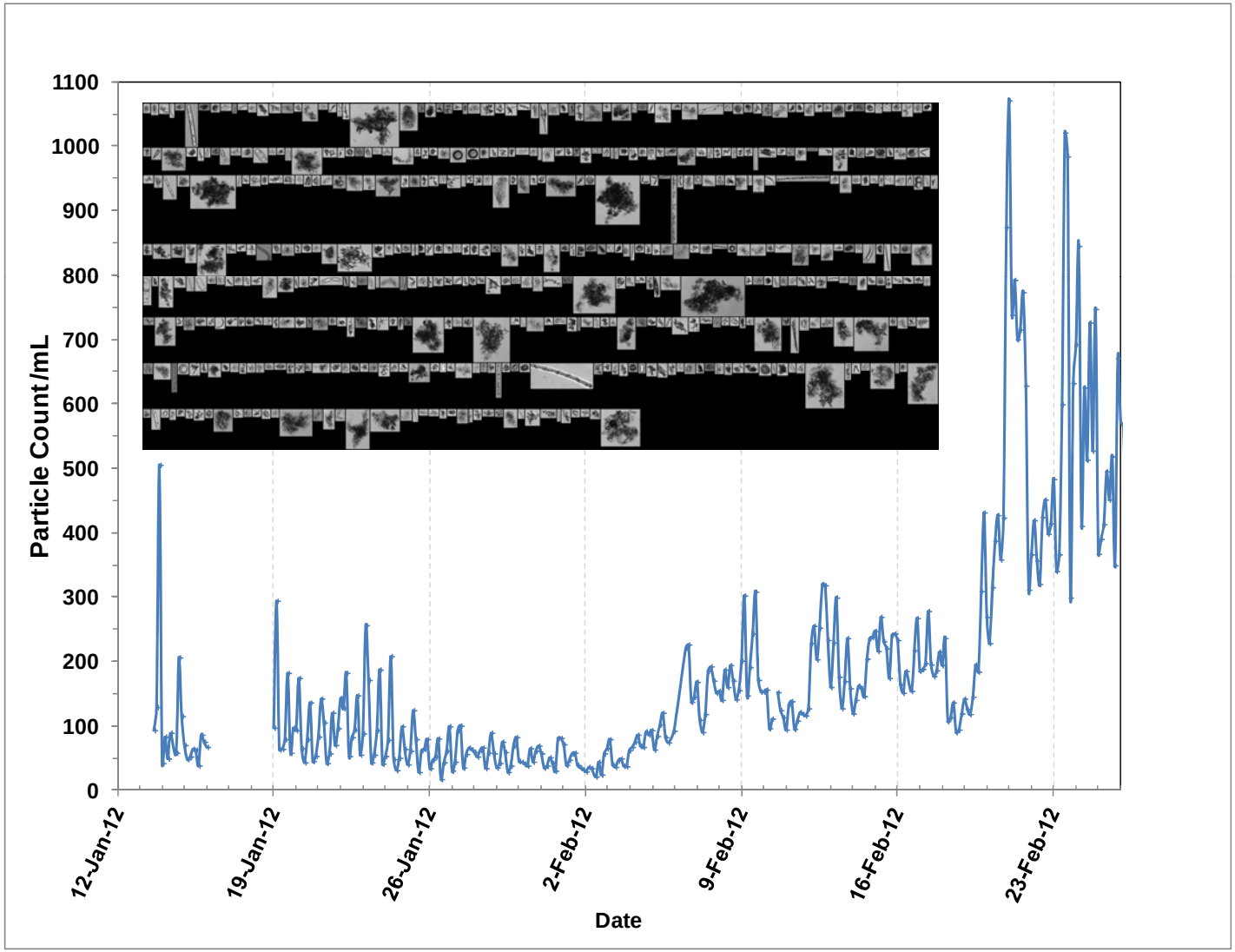




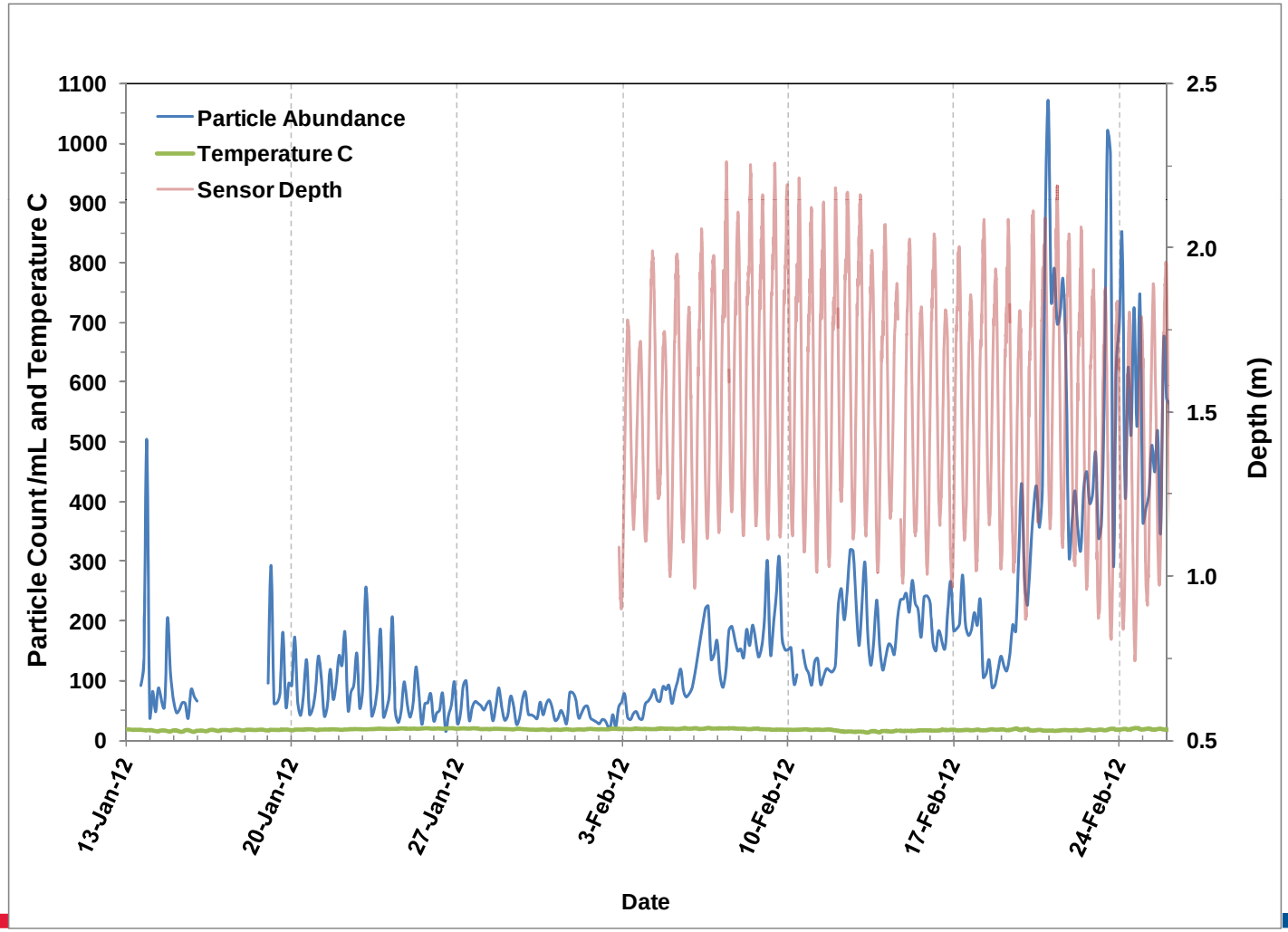
Environmental Characteristics During Deployment



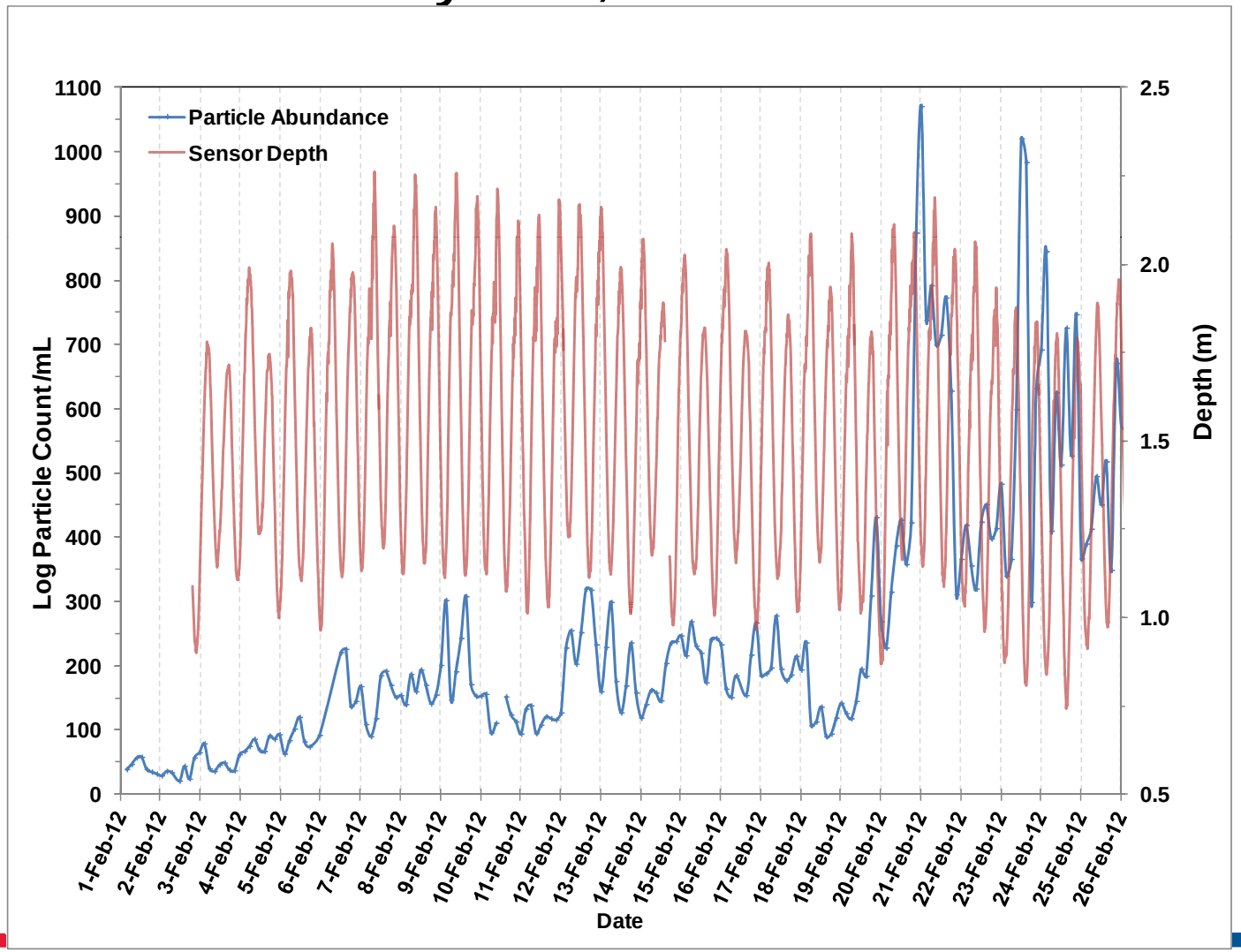
Total particle abundance Ponce Inlet, FL: January 12 through February 26, 2012



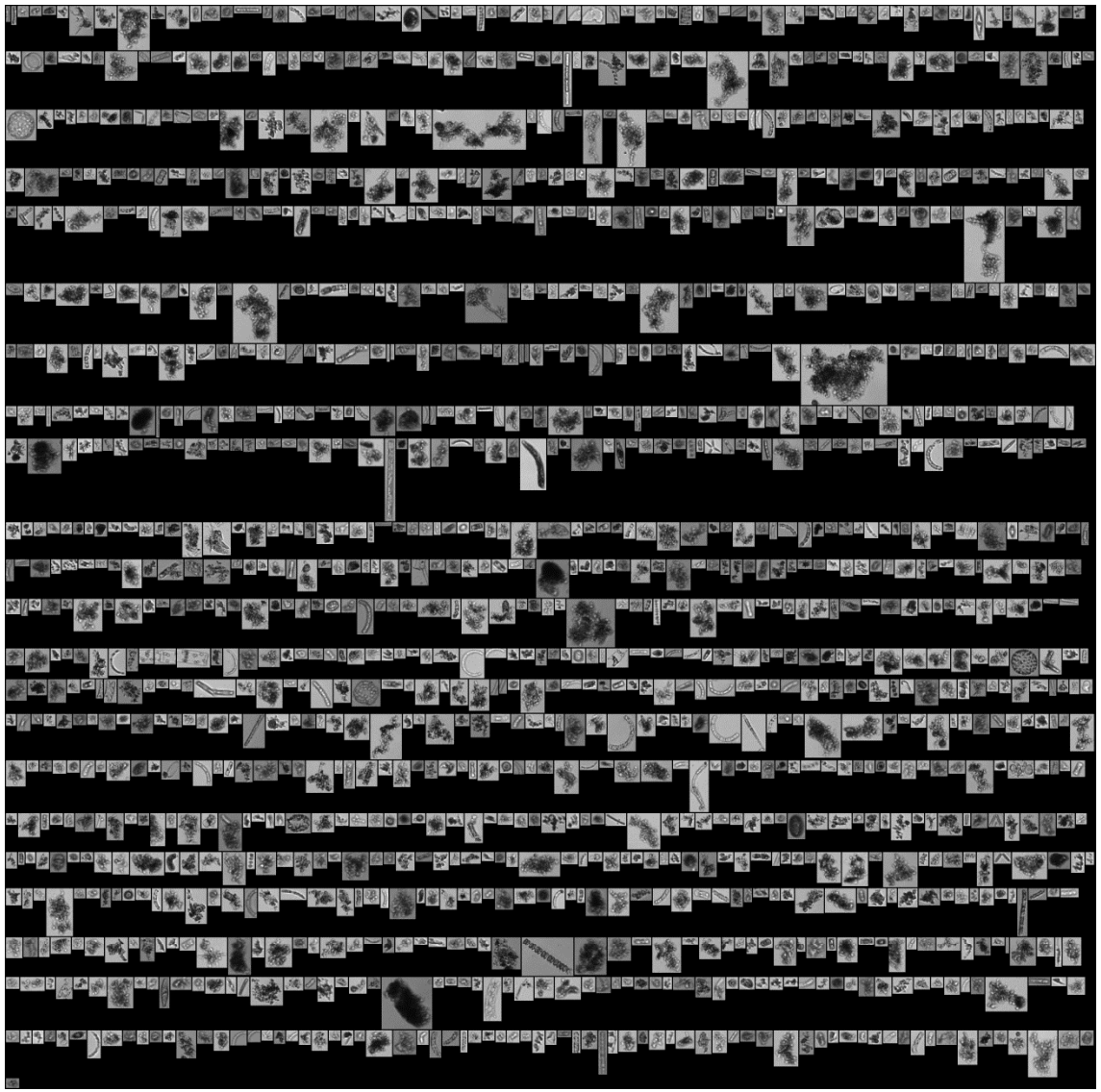
Total particle abundance, water temperature, and depth over SFC at Ponce Inlet, FL: January 12 through February 26, 2012



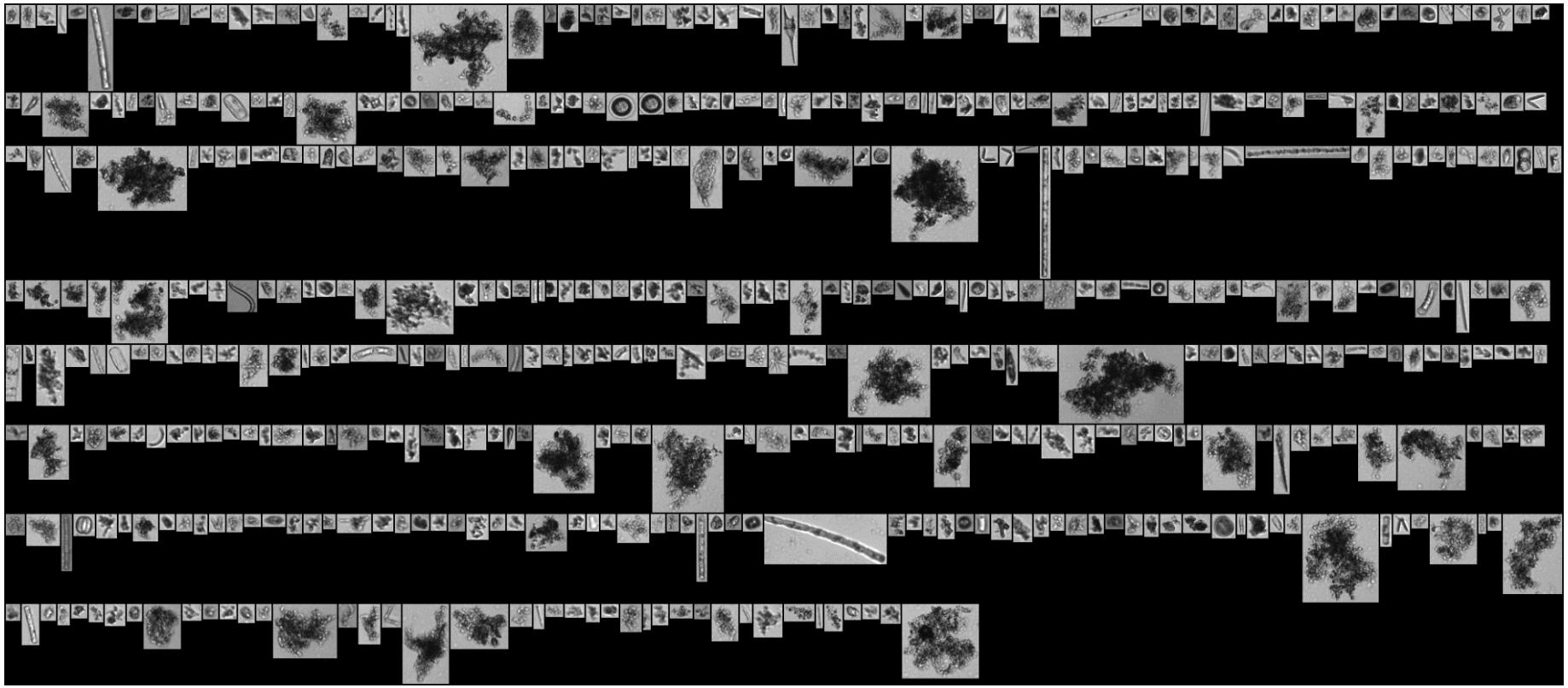
Total particle abundance and water depth over SFC at Ponce Inlet, FL February 1 through February 26, 2012



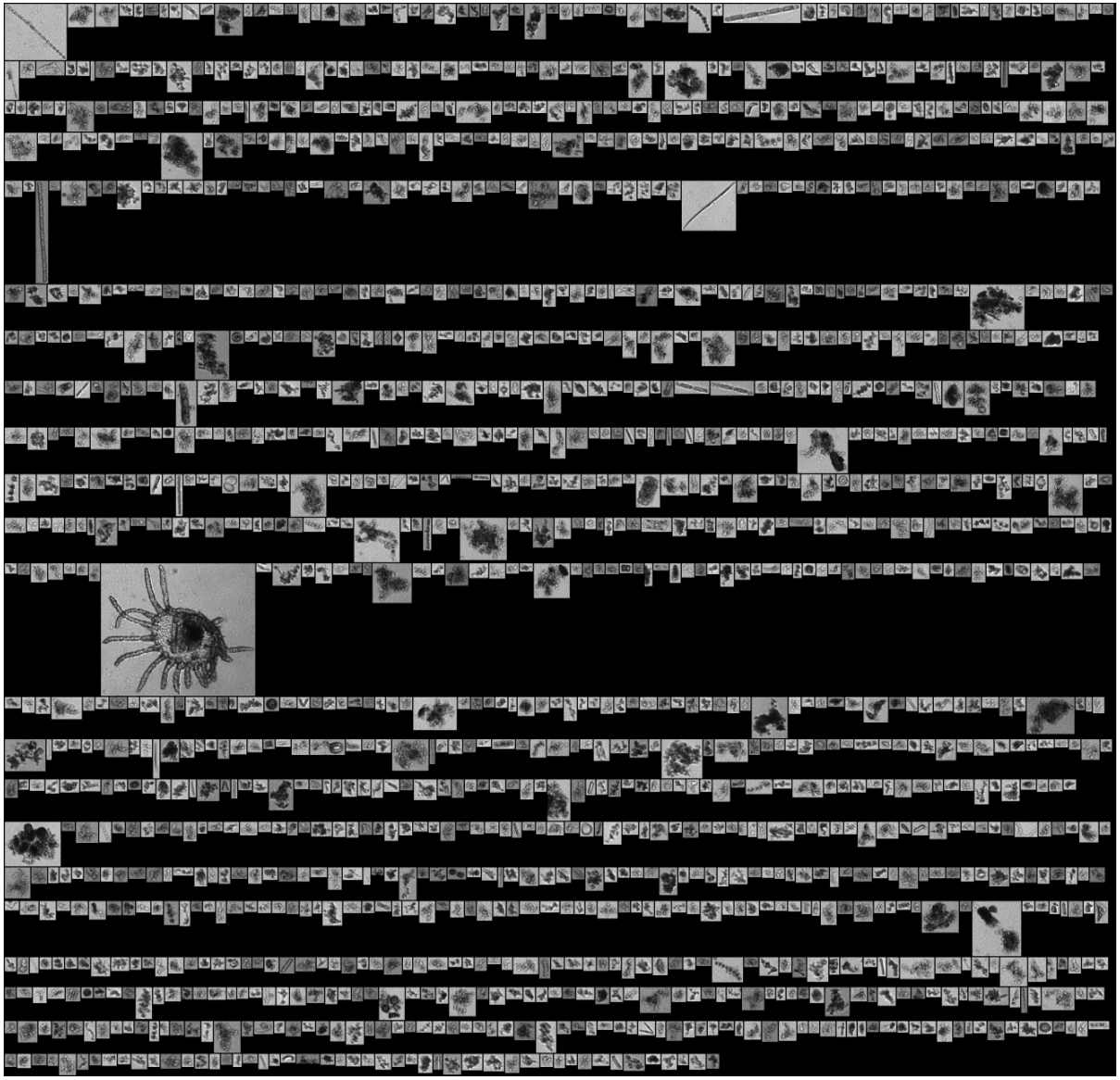
DAY 1 (1/13/2012 23:37)
129 Particles/ml



DAY 20 (2/1/2012 23:44)
35 Particles/ml



DAY 41 (2/22/2012 20:13)
451 particles/ml



DAY 43(2/24/2012 17:13)
513 particles/ml

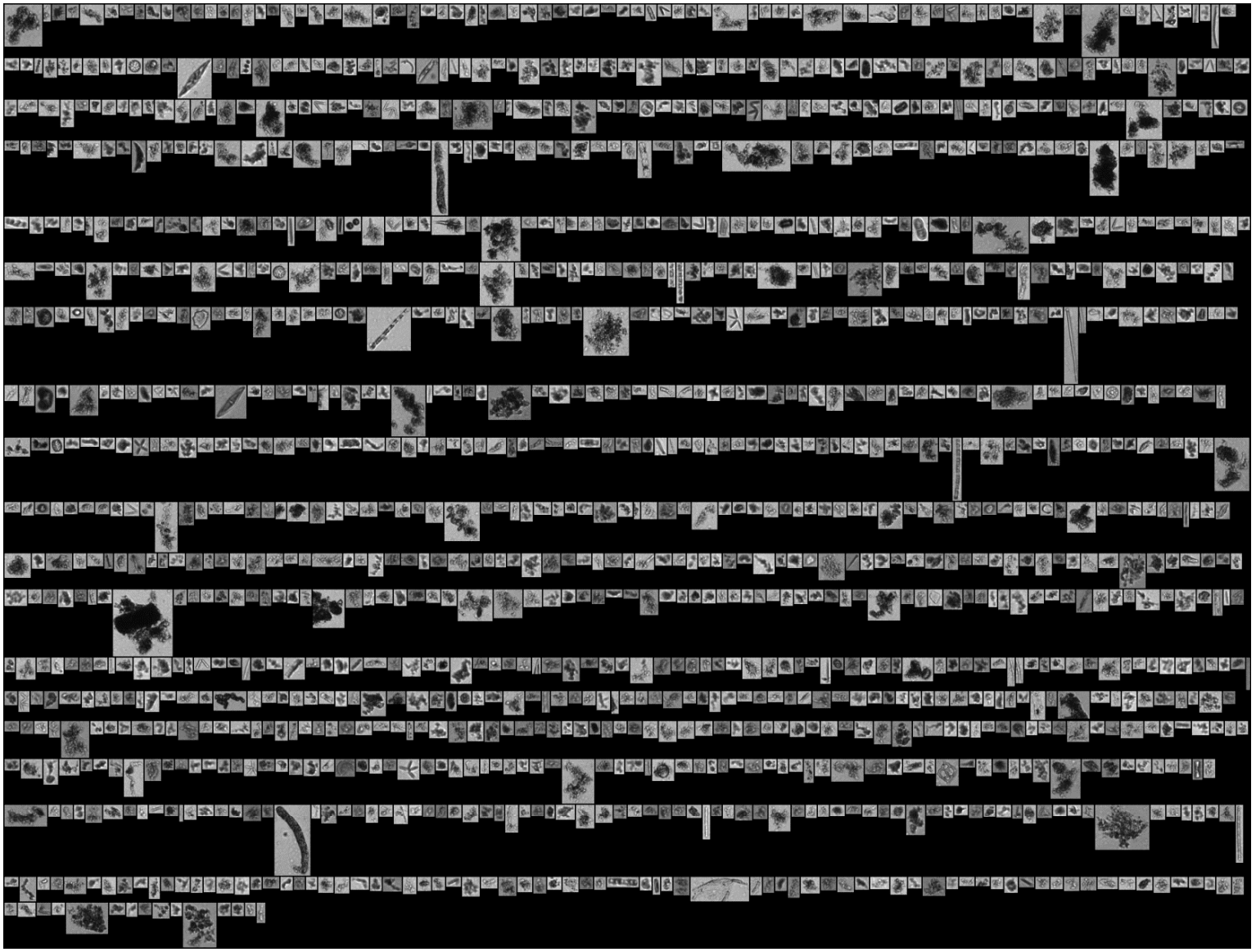
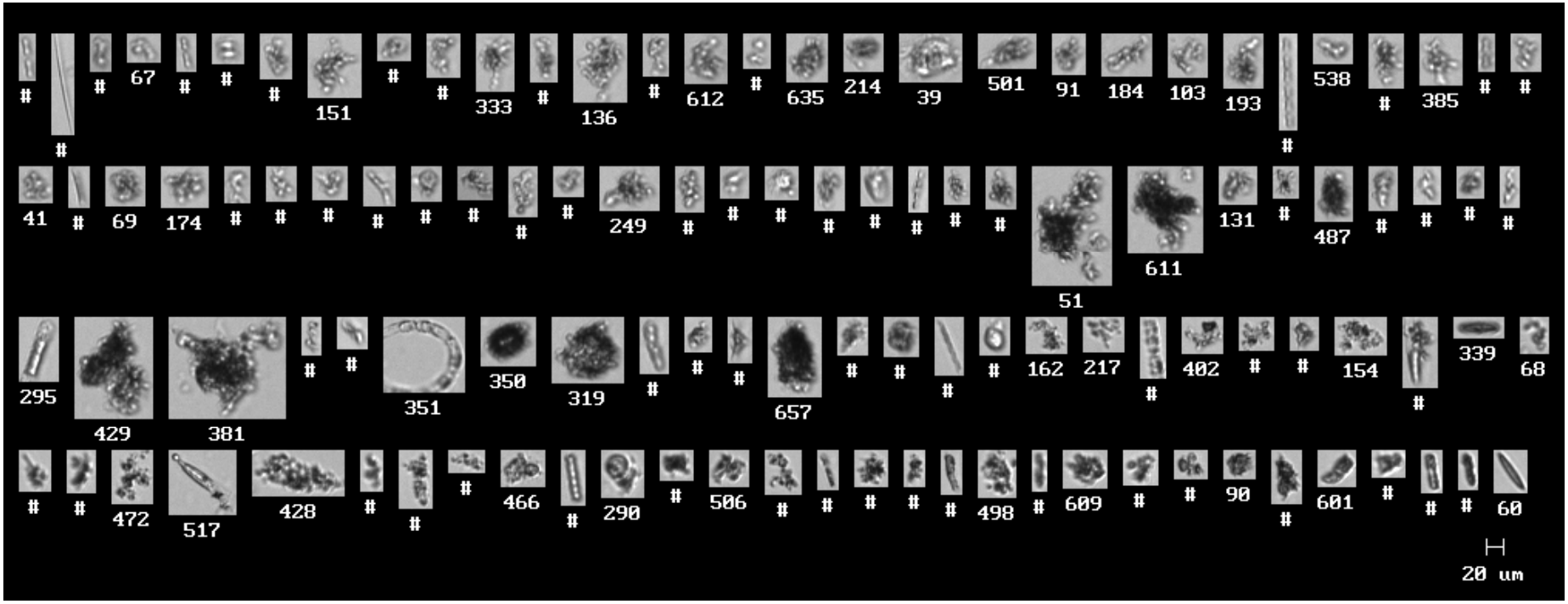
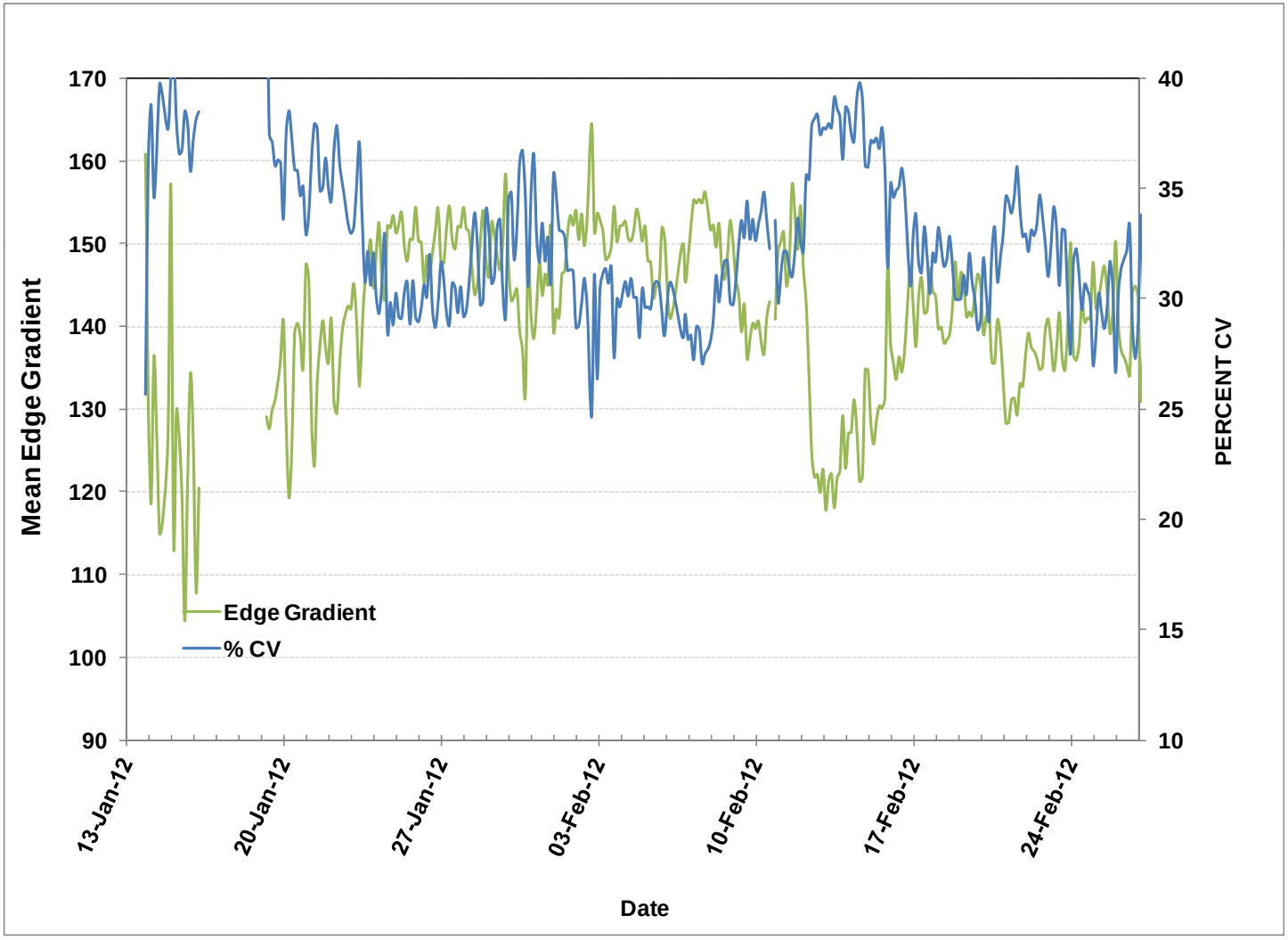


Image quality

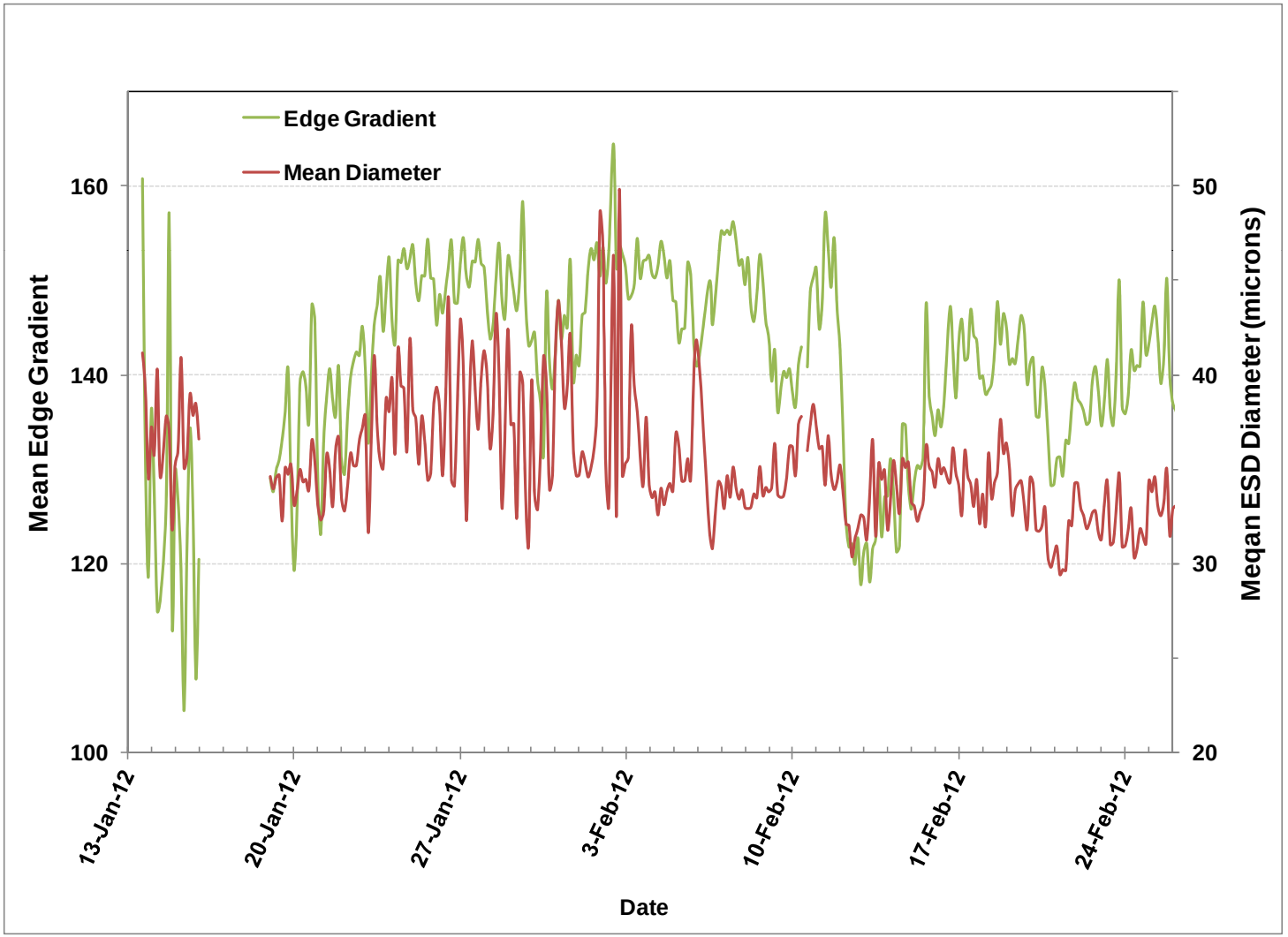
<u>Edge Gradient</u>: Average intensity of the pixels making up the outside border of a particle after an Edge Detect (Sobel) convolution filter has been applied to the raw camera image.



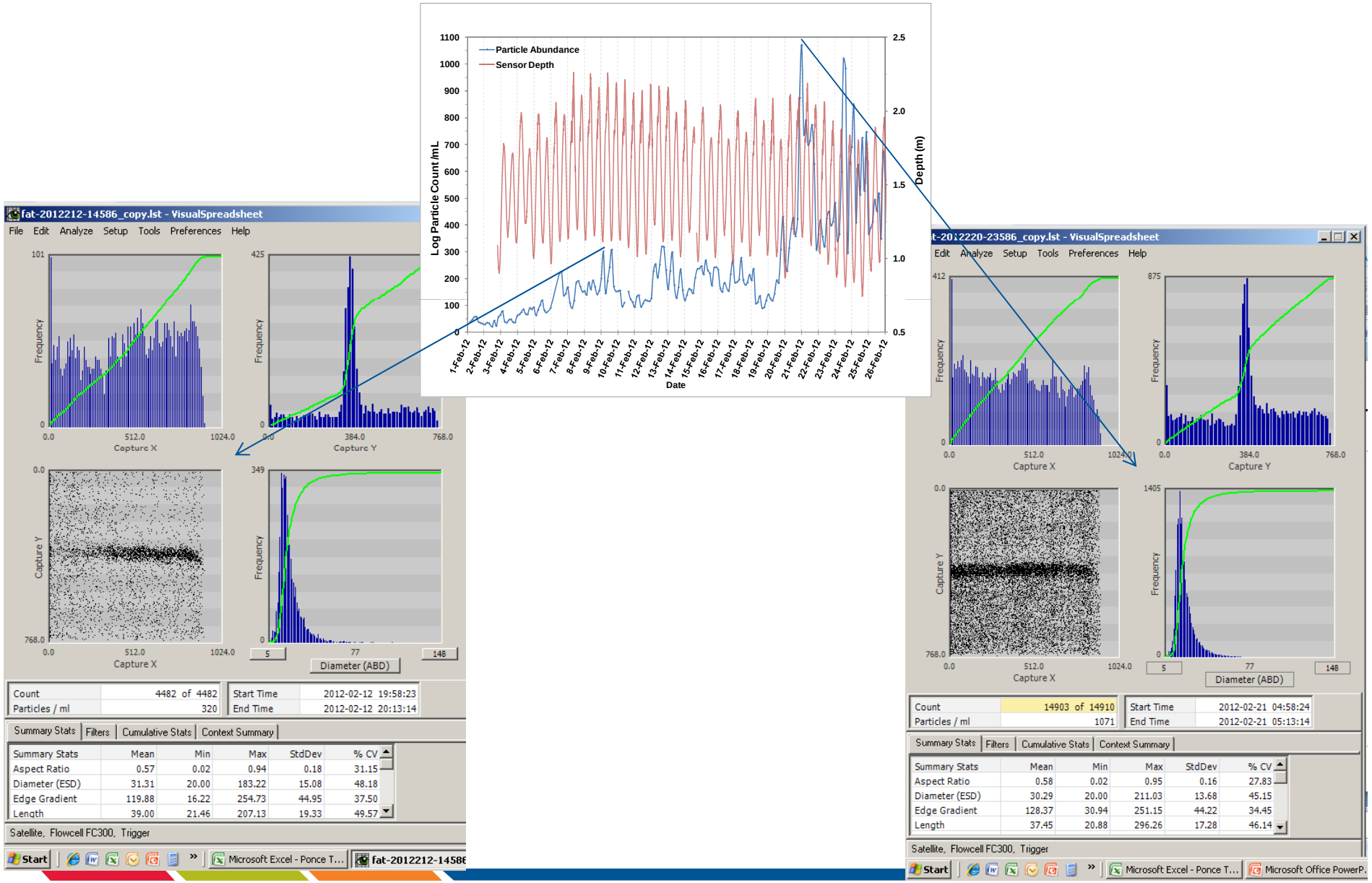
Mean edge gradient and percent coefficient of variation Ponce Inlet, FL, January 12 - March 2, 2012



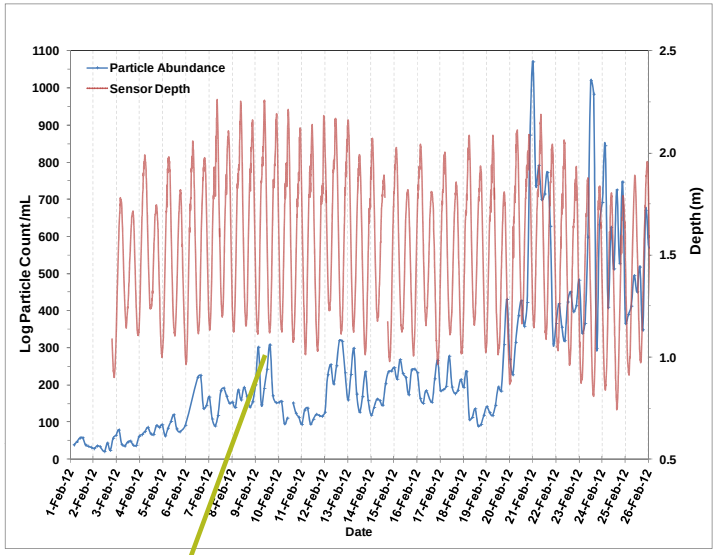
Mean particle diameter and edge gradient Ponce Inlet, FL, January 12 - March 2, 2012

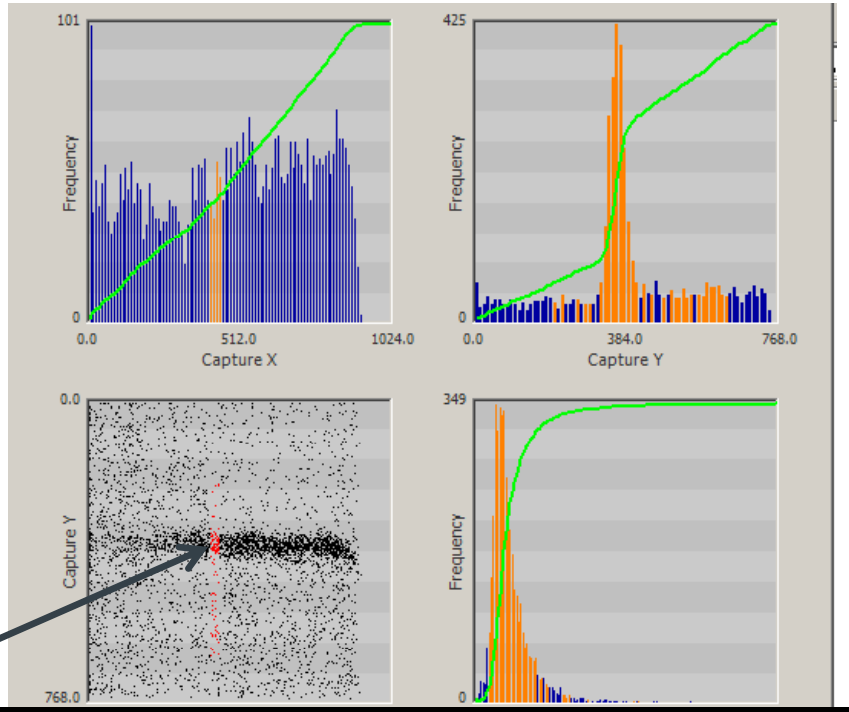


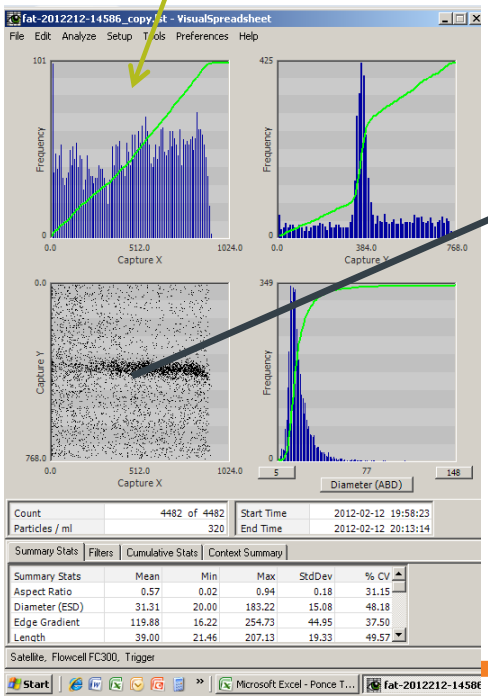
Data Processing

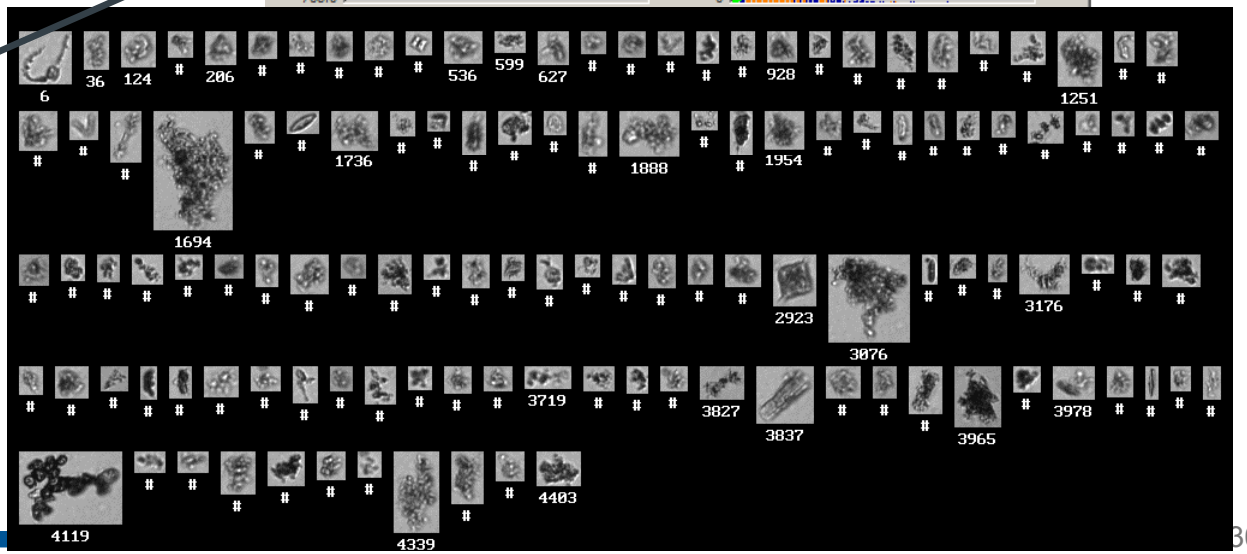


February 13, 2012 Peak Abundance

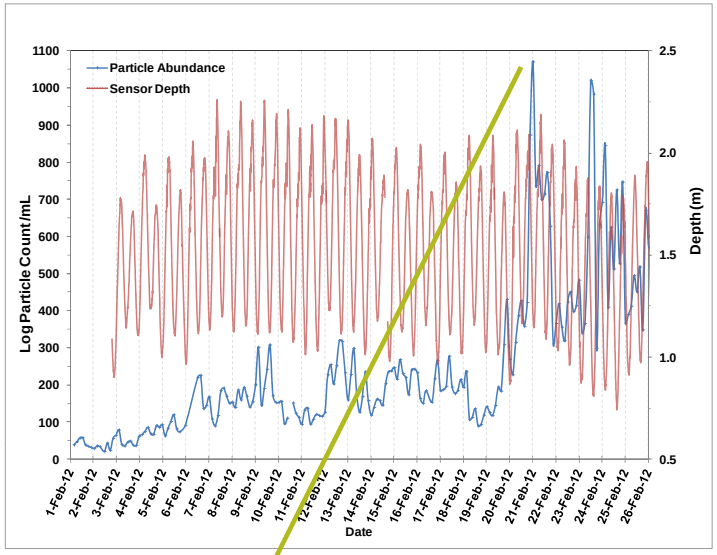


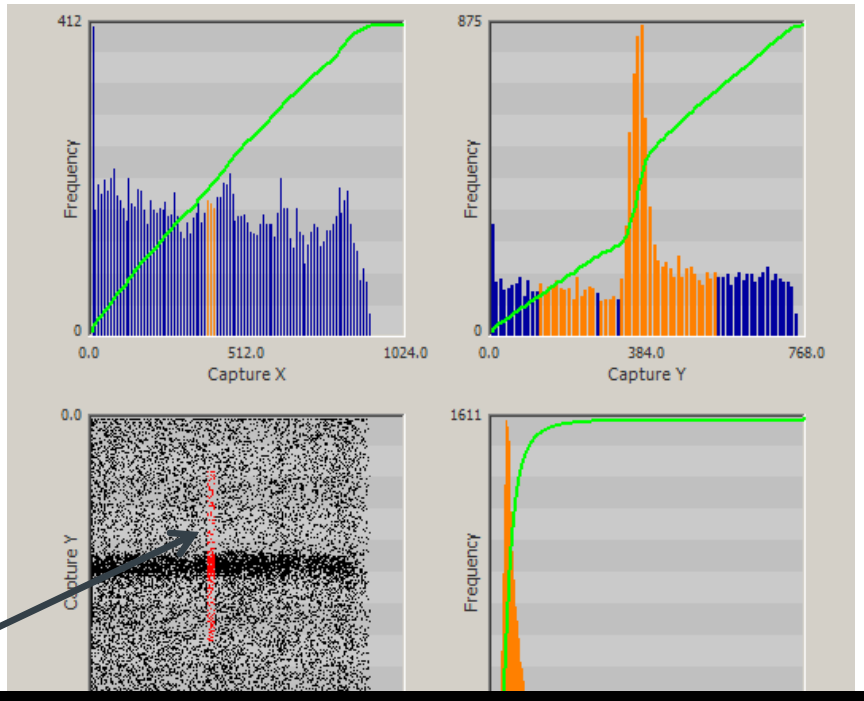


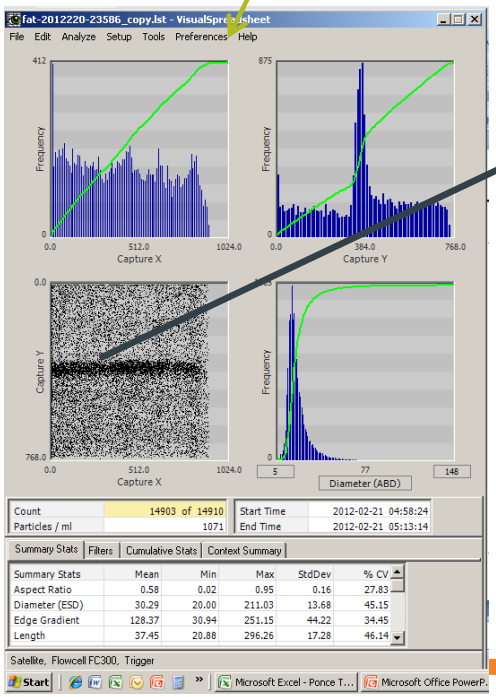


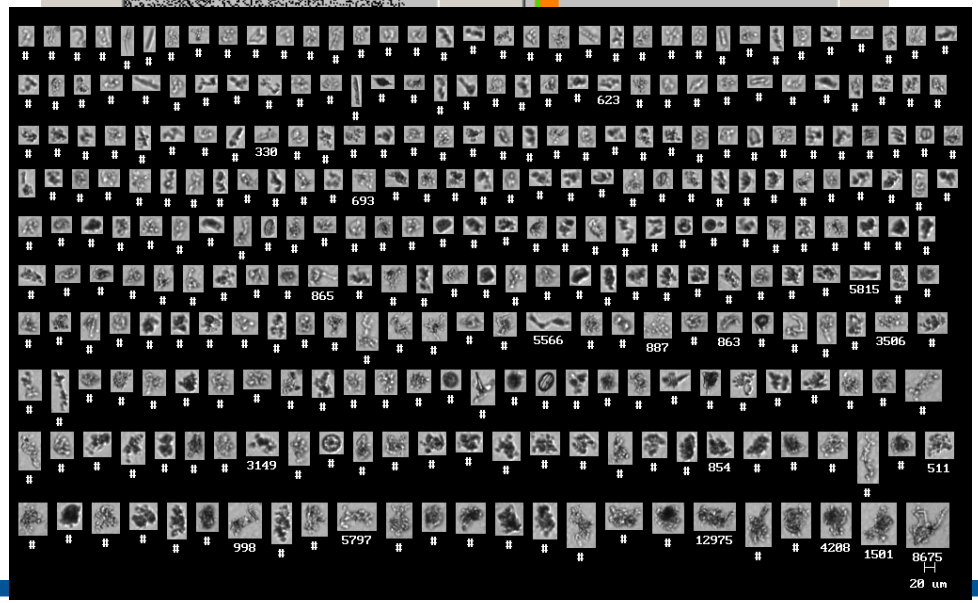


February 20, 2012 Peak Abundance



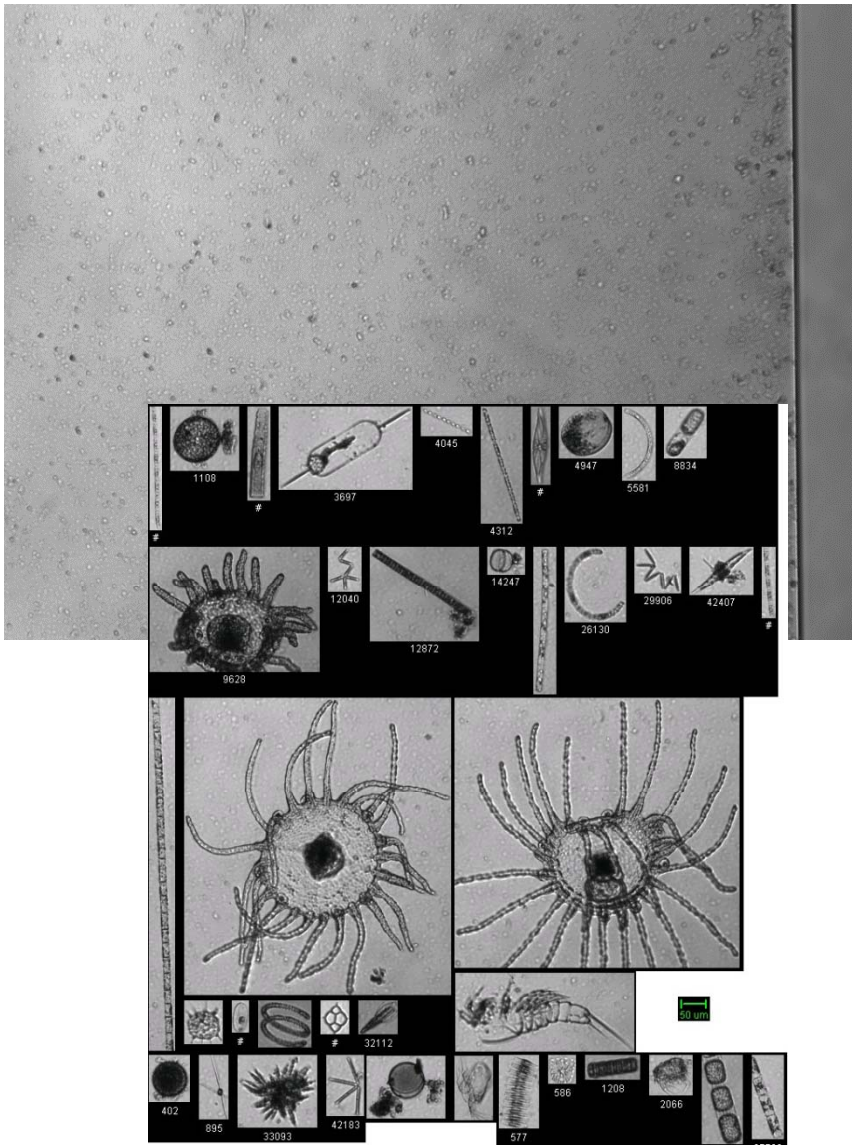






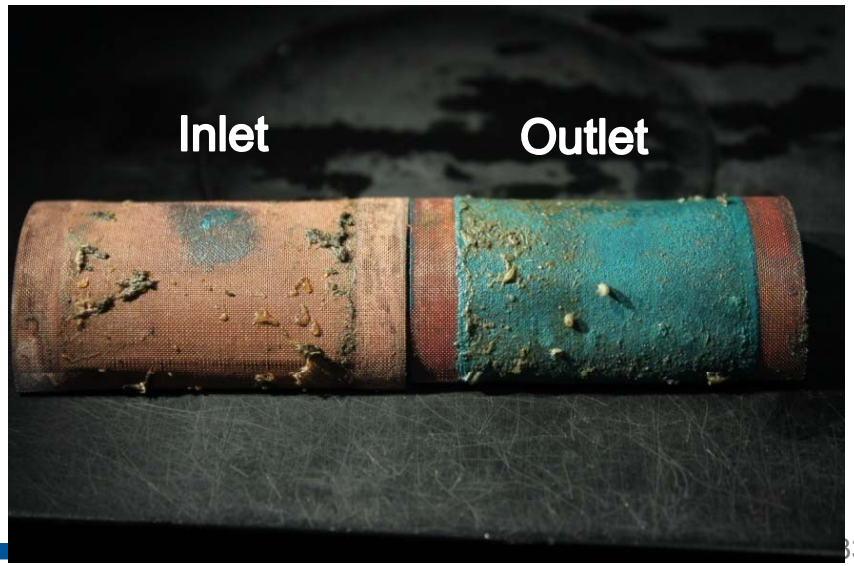
Conclusions
Field Endurance Test (Jan – Mar 2012)

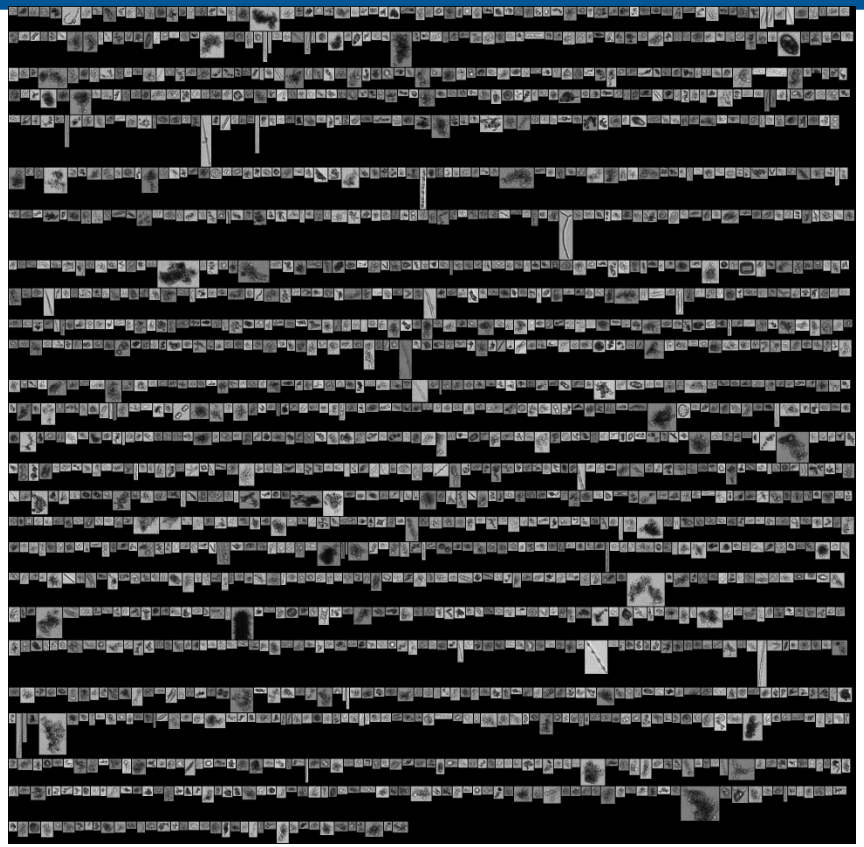
- **Controlled biofouling in flow cell for 50 days**
- Maintained image quality through end of test
- Maintained flow for 45 days
- **Corrosion protection adequate**

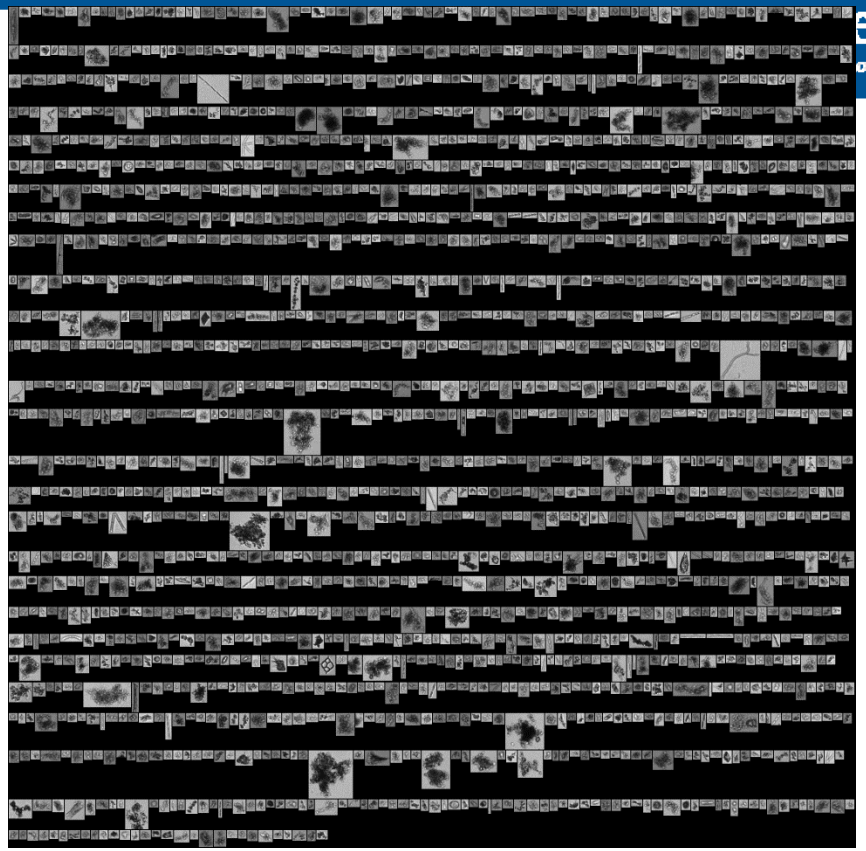


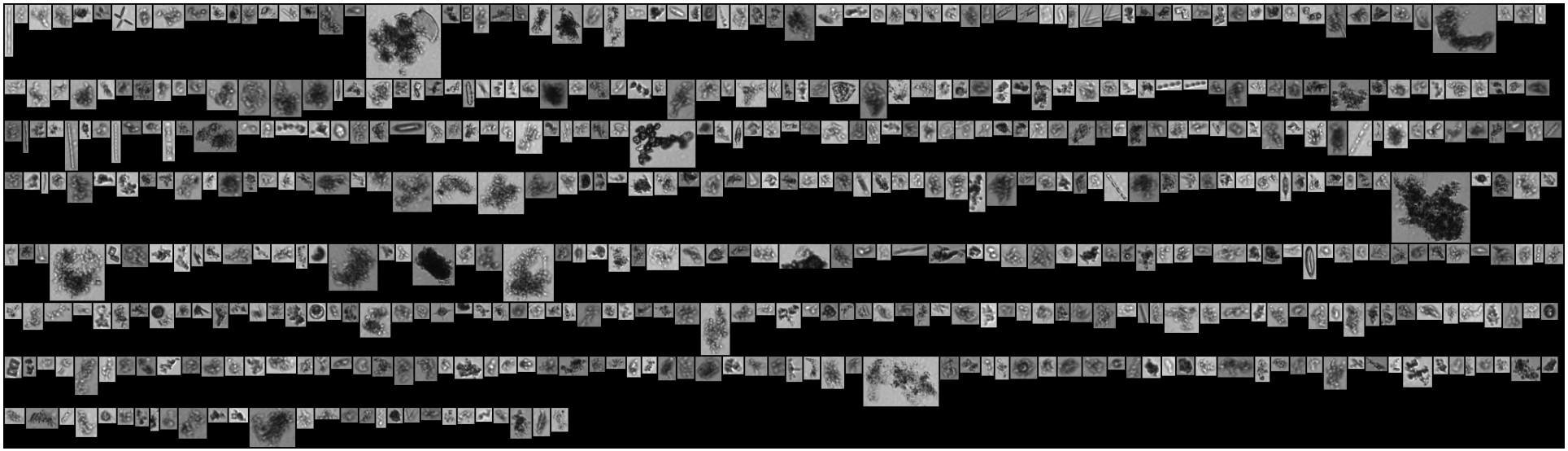
Biofouling control
Inlet and Outlet Filters

- Observed **loss of flow starting around Day 45**
- Inspection and post-mortum points to **corrosion on outlet filter as the likely cause**
- **Next steps**: Refurbish unit and address outlet screen corrosion









UV: System Implementation

- **Decision to implement UV was made based on biofouling control test results**

- Five UV LEDS
  - Concentrically arranged around the objective
  - Fully controllable on/off cycle
- Estimated UV dose: 250 mJ/cm^2