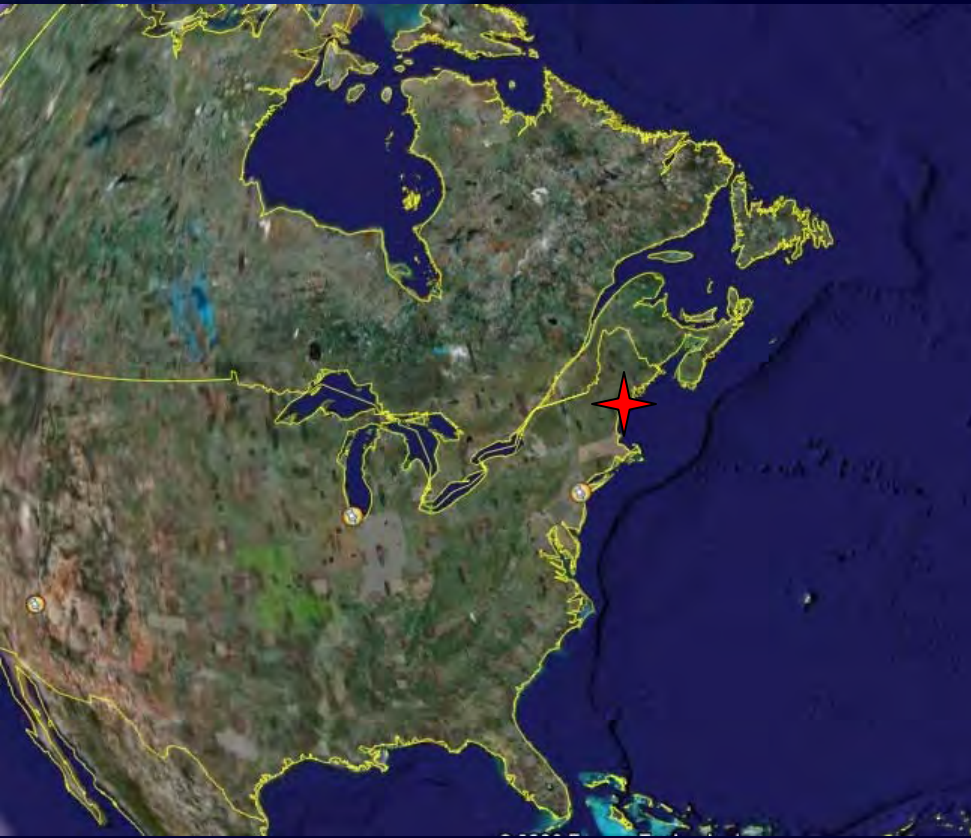


New methods for using a continuous imaging particle analyzer (FlowCAM®) for the analysis and classification of zooplankton

Harry Nelson
Director of Aquatic Sales & Marketing
Fluid Imaging Technologies

Fluid Imaging Technologies

- Founded – 1999
- Maine, USA (BLOS)
- **Flow Cytometer And Microscope (FlowCAM)**
- 300+ FlowCAMs sold
- **Product Development**
 - Depth-of-Focus Technology
 - VisualSpreadsheet 1.0, 2.4
 - Submersible FlowCAM



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Mar Ecol Prog Ser

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An imaging-in-flow system for automated analysis of marine microplankton

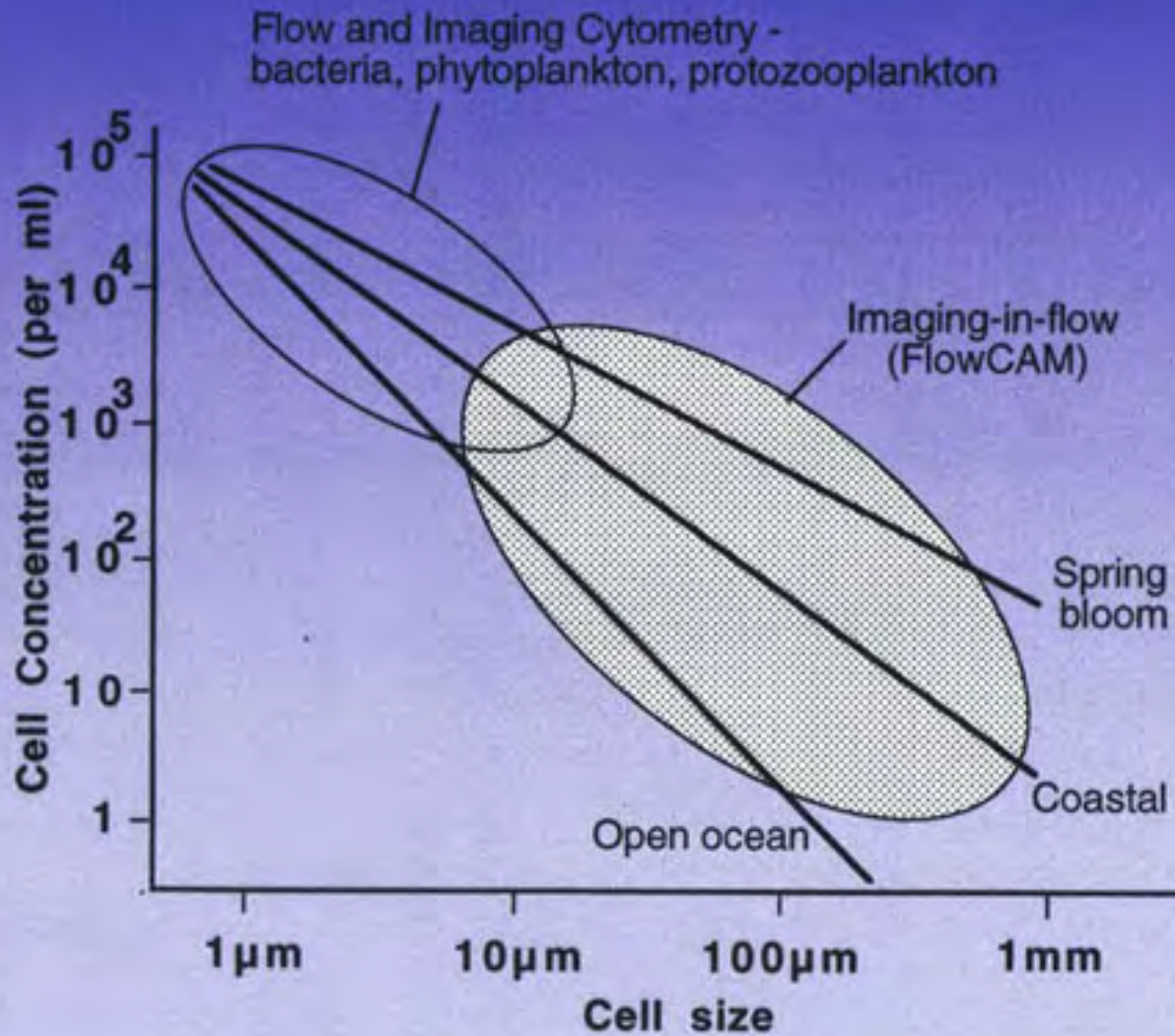
Christian K. Sieracki*, Michael E. Sieracki, Charles S. Yentsch

Bigelow Laboratory for Ocean Sciences, PO Box 475 McKown Point, West Boothbay Harbor, Maine 04575, USA

ABSTRACT: Present automated systems for counting and measuring marine plankton include flow cytometers and *in situ* plankton video recorders. Neither of these approaches are optimal for the microplankton cells which range in size from 20 to 200 μm and can be fewer than 10^4 l^{-1} . We describe here an instrument designed for rapid counting, imaging and measuring of individual cells and particles in the microplankton size range from cultures and natural populations. It uses a unique optical element to extend the depth of focus of the imaging lens, allowing a sample stream flow rate of 1 ml min^{-1} . The instrument stores a digital image of each particle along with real time fluorescence and size measurements. An interactive cytogram links a dot-plot of the size and fluorescence data to the stored cell images, allowing rapid characterization of populations. We have tested the system on live phytoplankton cultures and bead standards, proving the system counting and sizing accuracy and precision. The system provides images and size distributions for cultures or natural marine samples. It has been used successfully at sea to continuously monitor particles while underway. It may prove useful in studies of plankton community structure, ocean optics and monitoring for harmful algal species.

KEY WORDS: Imaging · Flow cytometer · Microplankton · Binary optical element · Cell counting · Cell sizing · Natural populations · High rate · Cultures

Plankton Size Spectra



FlowCAM Users



33 Countries (4 Pending)

FlowCAM Users



Eilat, Israel



Bigelow Labs



University of South Florida

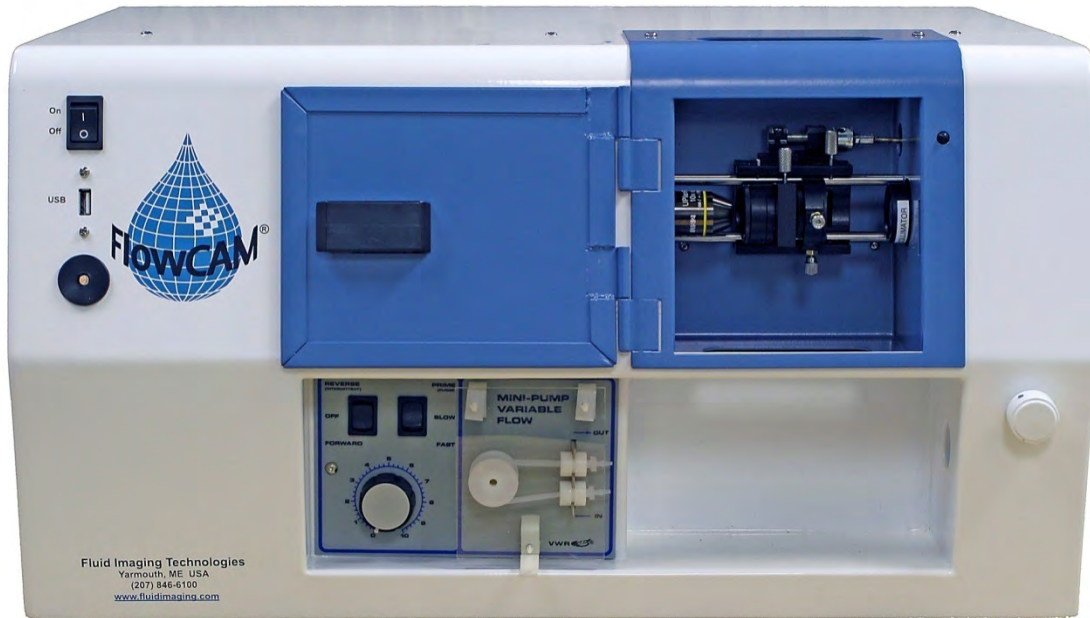


Univ Sao Paulo



Tara Oceans Project

FlowCAM Models



Bench Top



Portable

FlowCAM Models



Submersible FlowCAM

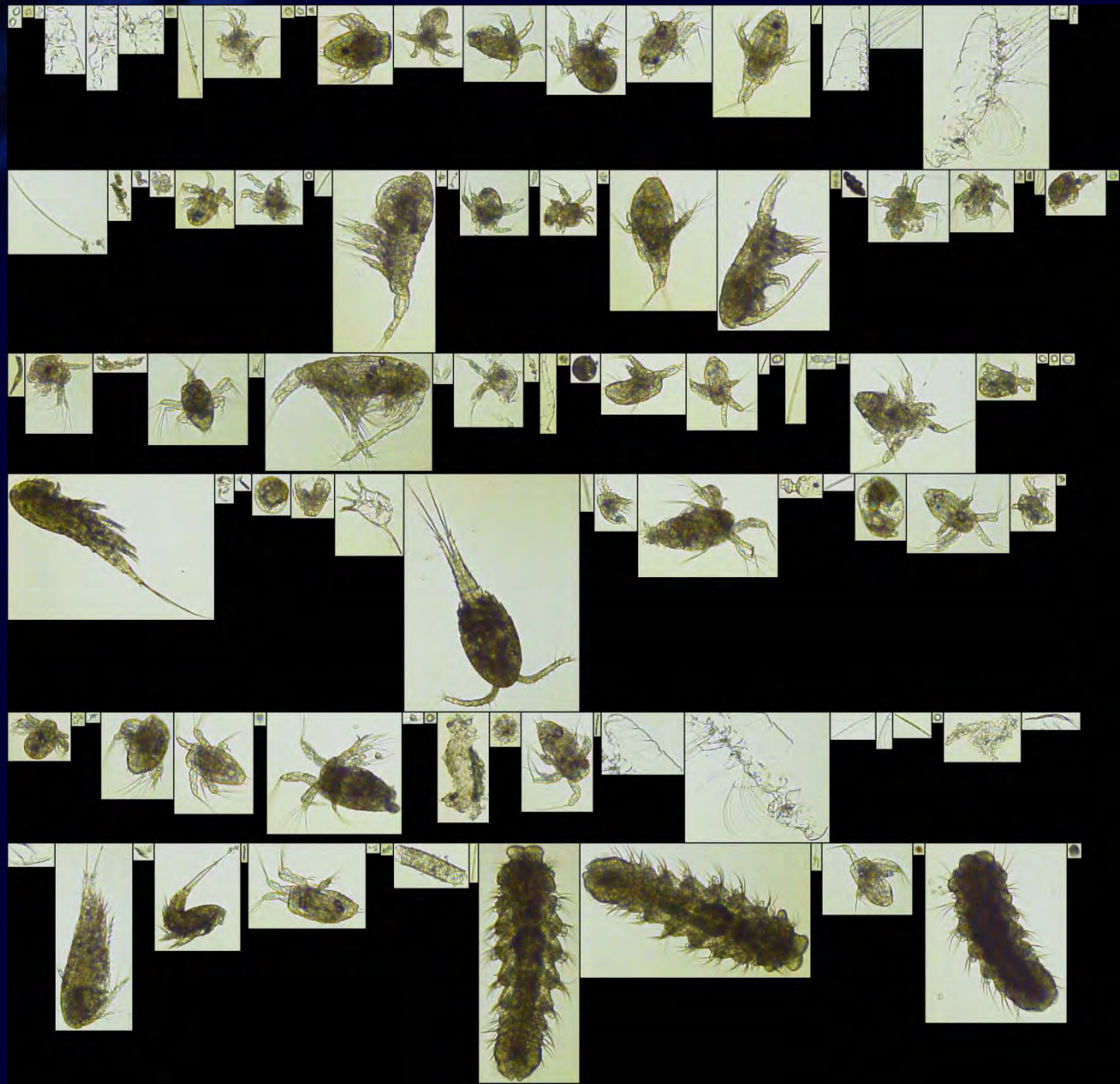
FlowCAM Specifications

- **Particle sizes –**
 - **Imaging – 3 μm to 2 mm**
- **Objectives – 20x, 10x, 4x, 2x**
- **Flow Cell – 50 μm to 2 mm**
 - **For Zooplankton “FOV” Flow Cell**
 - **300 μm , 1 mm, and 2 mm**
- **Processing Capability –**
 - Flow - .1 ml/min to 12 ml/min**
 - Density – 5,000,000 particles/ml (*Auto Trigger*)**

FlowCAM Features

- Continuous imaging (1-20 frames/sec)
- Can be used in laboratory or *in-situ*
- Size *and* shape info for all particles/cells
- Multiple Methods of Image Acquisition
 - 2 Channel Fluorescence/Scatter detection
 - Continuous imaging (1-20 frames/sec)
- *VisualSpreadsheet*®
 - 30+ Image Parameters collected and measured
 - Image Recognition
 - Automated Identification, Classification & Enumeration
- Image Collages exportable to Zoo/PhytoImage

Raw TIF File



FlowCAM Applications

- **Aquatic Plankton Research**
 - **Discreet or Continuous Sampling**
 - **In Lab**
 - **Cruises**
 - *In situ*
- **HAB Monitoring**
- **Culture Studies**
- **ZM/QM Veliger Detection & Enumeration (Cross Polarization)**
- **Water Treatment (Drinking)**
 - **Plankton Monitoring – T&O and Cyanobacteria**
 - **Particle Removal Analysis**
- **Ballast Water Testing & Research**
 - **Viability Analysis**
- **Algae-to-BioFuels**
- **Training and Education**

FlowCAM Applications (Industrial)

➤ **Pharmaceutical**

- Parental Drugs, Drug Formulations
- 21 CFR Compliant

➤ **Food**

- Particle Size & Shape Analysis

➤ **Yeast Analysis**

➤ **Abrasives**

- Particle Size & Shape Analysis

➤ **Chemical**

- On-line Quality Control and Research

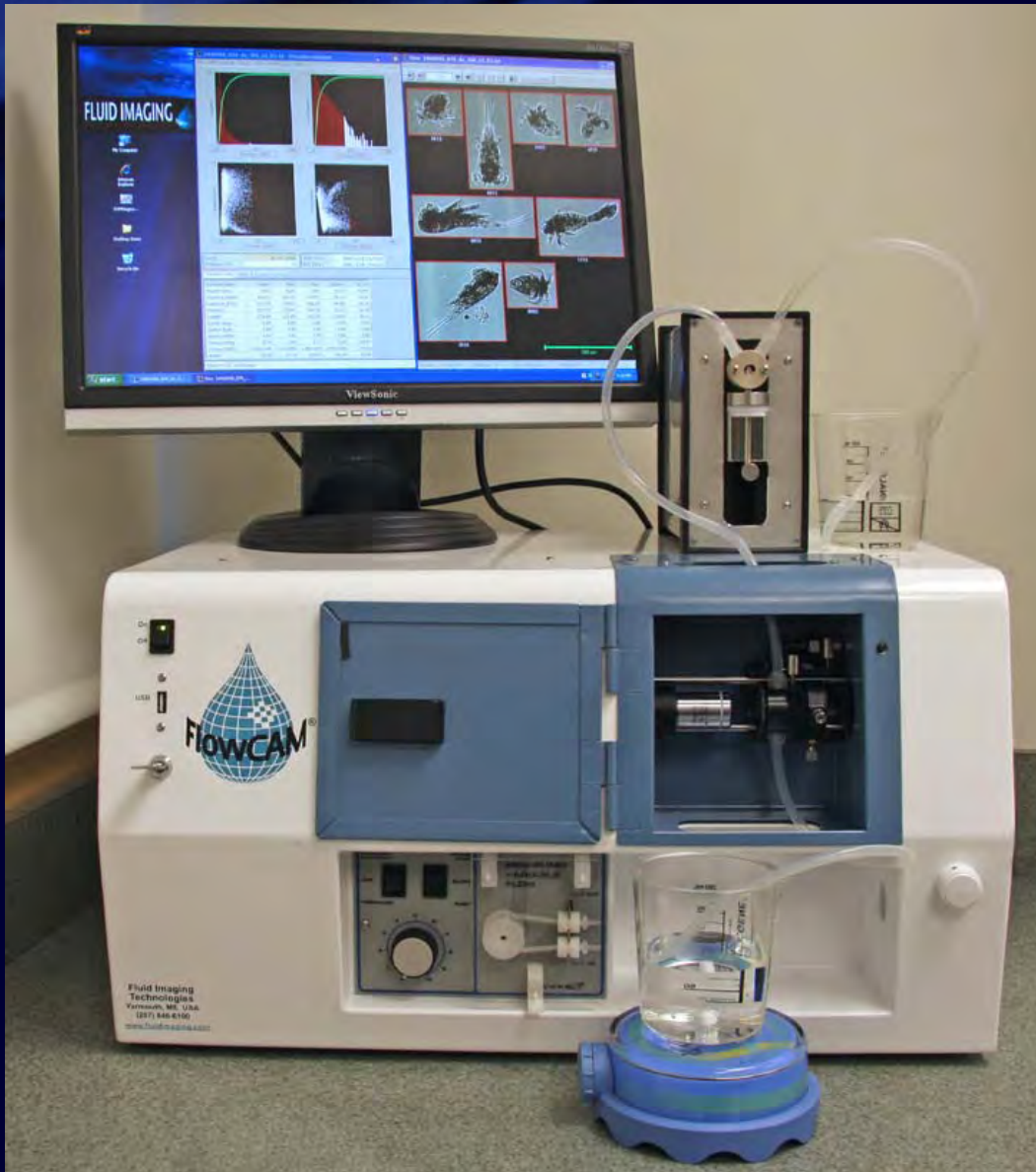
➤ **Algae-to-BioFuels**

- Lipid Analysis (Nile Red), Counting, Bio Reactor Contamination

Zooplankton Challenges

- Large particles settle quickly
- Large particles flow outside camera FOV
- Agglomeration of particles
(esp. fixed samples)
- Camera view area (4.2 mm x 3.6 mm)
- Two dimensional image/Particle orientation

Zooplankton Set-up



- 1 or 2 mm 'FOV' Flow Cell
- Draw sample from beaker With Stir Bar
- Syringe Pump
- Draw sample vertically
- Add surfactant to sample

Integrated Syringe Pump

Context [X]

Reports Notes Run Summary Stop PMT/Scatter
Load Capture Flow Cell Fluidics Camera Filter

Settings

Sample volume ml (syringe size 1.00 ml)
Flow rate ml/min (0.010 to 20.000)
Autolmage Rate frames per second

Estimated Efficiency and Run Time

Efficiency percent *Warning: recommended efficiency exceeded*
Run Time minutes

Priming

Priming Method:

- machine prime
- manual prime with sample
- manual prime with non-sample

Sample Dilution

The sample fluid was diluted or concentrated


Dilution ratio

OK Cancel

View 068-090036_copy.lst

File Edit Sort Filter Statistics Show

1 of 1 Z+ Z- Z1 Show All



81

100 um

Particle ID	81
Area (ABD)	57669
Aspect Ratio	0.55
Average Blue	72.70
Average Green	93.14
Average Red	96.67
Circle Fit	0.00
Compactness	32.87
Convex Perimeter	1910.94
Diameter (ABD)	270.97
Diameter (ESD)	602.86
Edge Gradient	0.00
Elongation	101.24
Filter Score	0.00
Length	734.65
Perimeter	5005.84
Sigma Intensity	41.56
Sum Intensity	2.63e+006
Transparency	0.55
Volume (ABD)	1.04e+007
Volume (ESD)	1.15e+008
Width	406.27

Saved Total: 83 Selected: 1 Sort: Length Classified: 0

33 Image Parameters

Visual Spreadsheet

Automated Recognition Algorithm

Field Values

Name	Aspect Ratio
Filter Min	0.20
Filter Max	0.80
Data	
Min	0.19
Max	0.80
Mean	0.57
Std Dev	0.18

- Determine Min/Max and Mean of Parameter
- Develop scale using Std Dev of the particle parameter as the base to 'Normalize Values' of Attribute – $(u_i - x_i) / \sigma_i$
- Calculates Image 'Filter Score' based on 'Distance' parameters are from Normalized Means
- Classifies Images based on Filter Score
- Repeat, in order, for next Class



606



231



1499



1171

Binary Image




467

View 075-102922.lst

File Edit Sort Filter Statistics Show

1 of 1 Z+ Z- Z1 Show All



467

Particle ID	467
Area (ABD)	472459
Aspect Ratio	0.63
Circle Fit	0.00
Compactness	45.23
Convex Perimeter	5975.27
Diameter (ABD)	775.60
Diameter (ESD)	1890.72
Edge Gradient	84.60
Elongation	140.07
Filter Score	0.00
Intensity	64.19
Length	2320.65
Perimeter	16683
Roughness	2.79
Sigma Intensity	70.35
Transparency	0.59
Volume (ABD)	2.44e+008
Volume (ESD)	3.54e+009
Width	1464.97

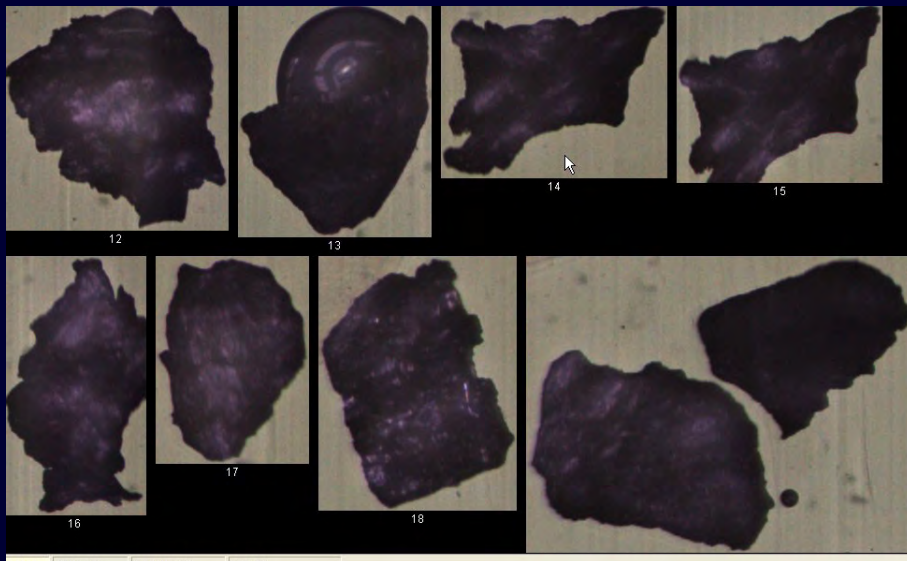
1000 um

Modified Total: 516 Selected: 1 Sort: ID Classified: 0

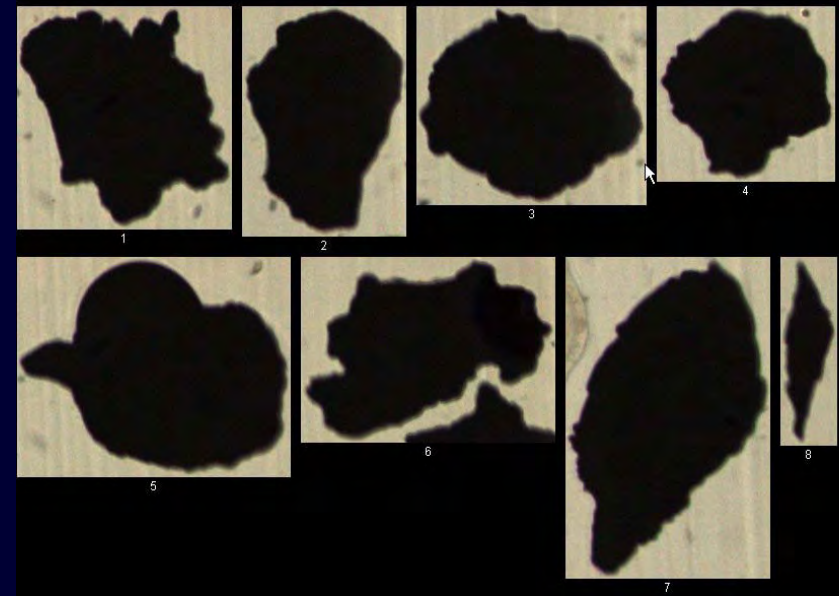
FlowCAM

Moving Forward

- 1/2 X Magnification
- Front Light LED Array



Front Light



Back Light

FlowCAM

Moving Forward



467

Back Light



Front Light

VisualSpreadsheet™

Moving Forward

C. Add new particle properties to VisualSpreadsheet

- First develop a particle property test set of images with many 'standard' shapes.
- Add filled area and shape properties based on perimeter and filled area.
- Add alternative size measurements such as Martin diameter and chord lengths.
- New BioVolume Formulas (Cylinders, Rods, etc.)
- Add convexity measures based on perimeter and on area measures.

D. Develop new library/classifier infrastructure

- Method to **share** training sets, e.g., using libraries in their current form.
- Create a classification generator, probably outside VisualSpreadsheet, that builds a classifier from some training sets. This generator will use OpenCV.
- The classification generator will include standard methods to create and test classifiers including cross-validation and display of confusion matrices; the user should be able to click on the cells in the confusion matrix to see the particle images for that cell.
- Output of the classification generator will be classifiers that can be used within VisualSpreadsheet just as filters are now used.

Thanks To:

Fluid Imaging Technologies

Matt Duplisea – Manager of Engineering

Ben Spaulding – Laboratory Manager

Bob Grimm – Senior Electronics Engineer

