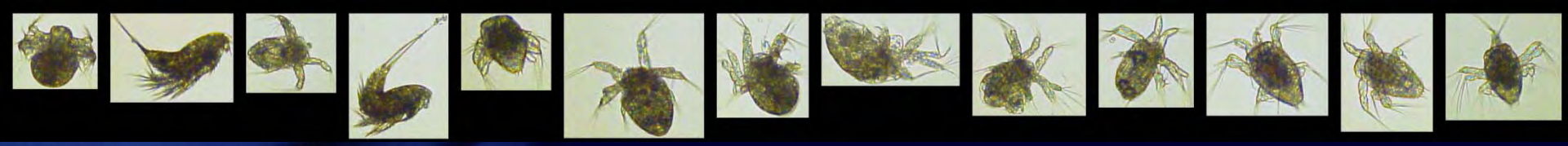

New methods for using a continuous imaging particle analyzer (FlowCAM®) for the analysis and classification of zooplankton

Harry Nelson
Director of Aquatic Sales & Marketing
Fluid Imaging Technologies

FLUID IMAGING TECHNOLOGIES

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

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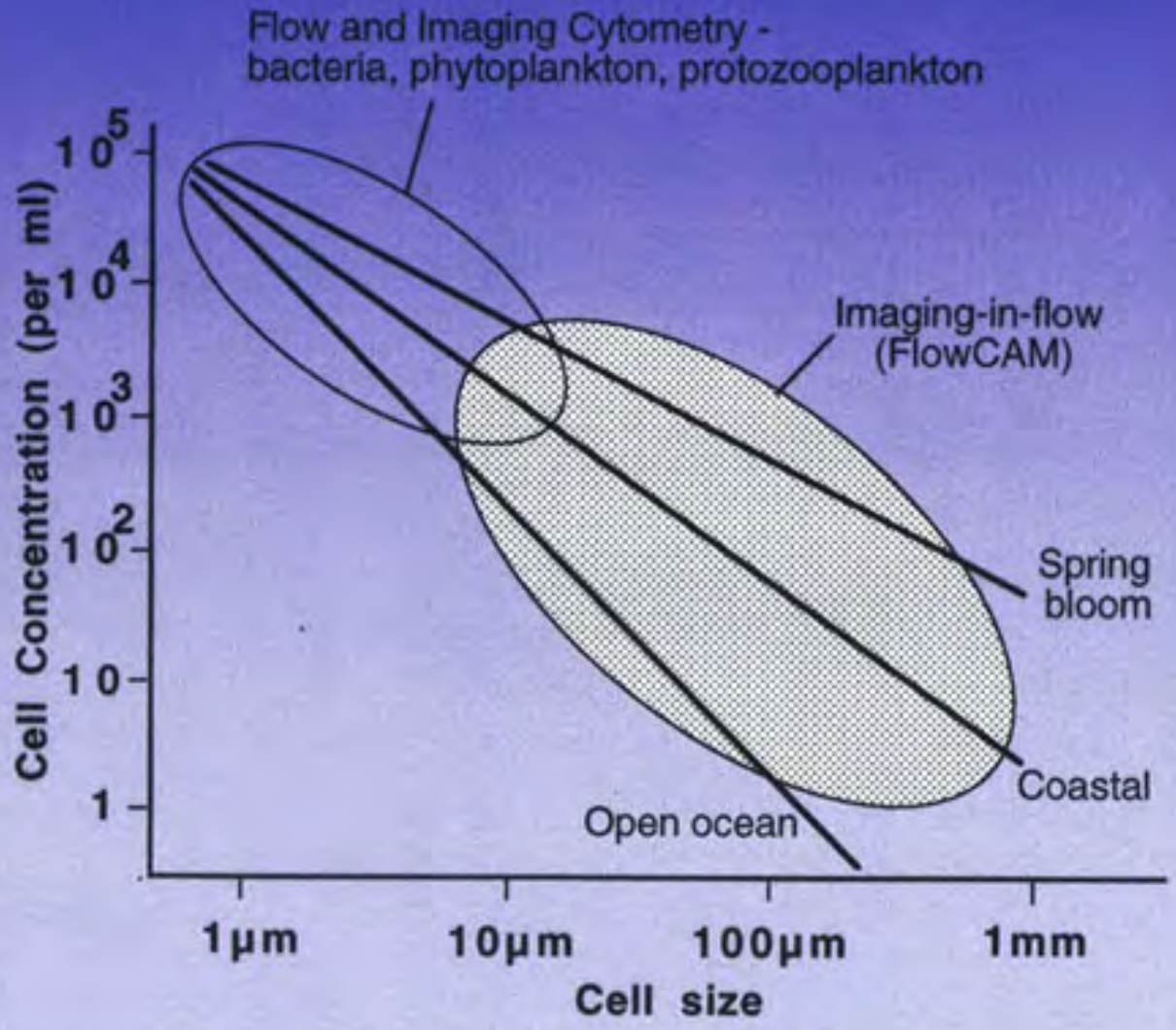
ABSTRACT: Present automated systems for counting and measuring marine plankton include flow cytometers and *in situ* plankton video recorders. Neither of these approaches are optimal for the microplankton cells which range in size from 20 to 200 µm and can be fewer than 10^4 l^{-1}. We describe here 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

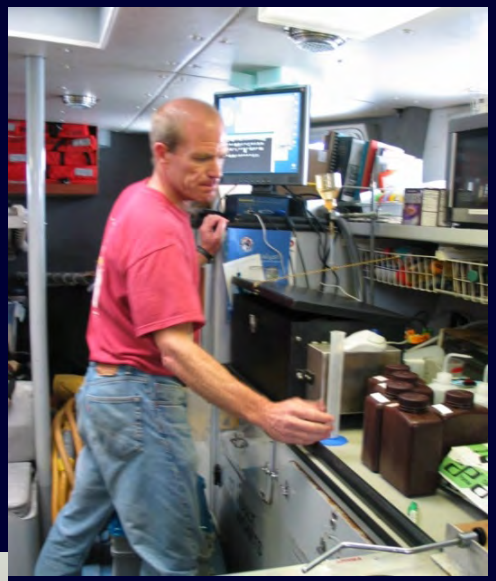


FlowCAM Users

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
    - 300µm, 1 mm, and 2 mm
- Processing Capability –

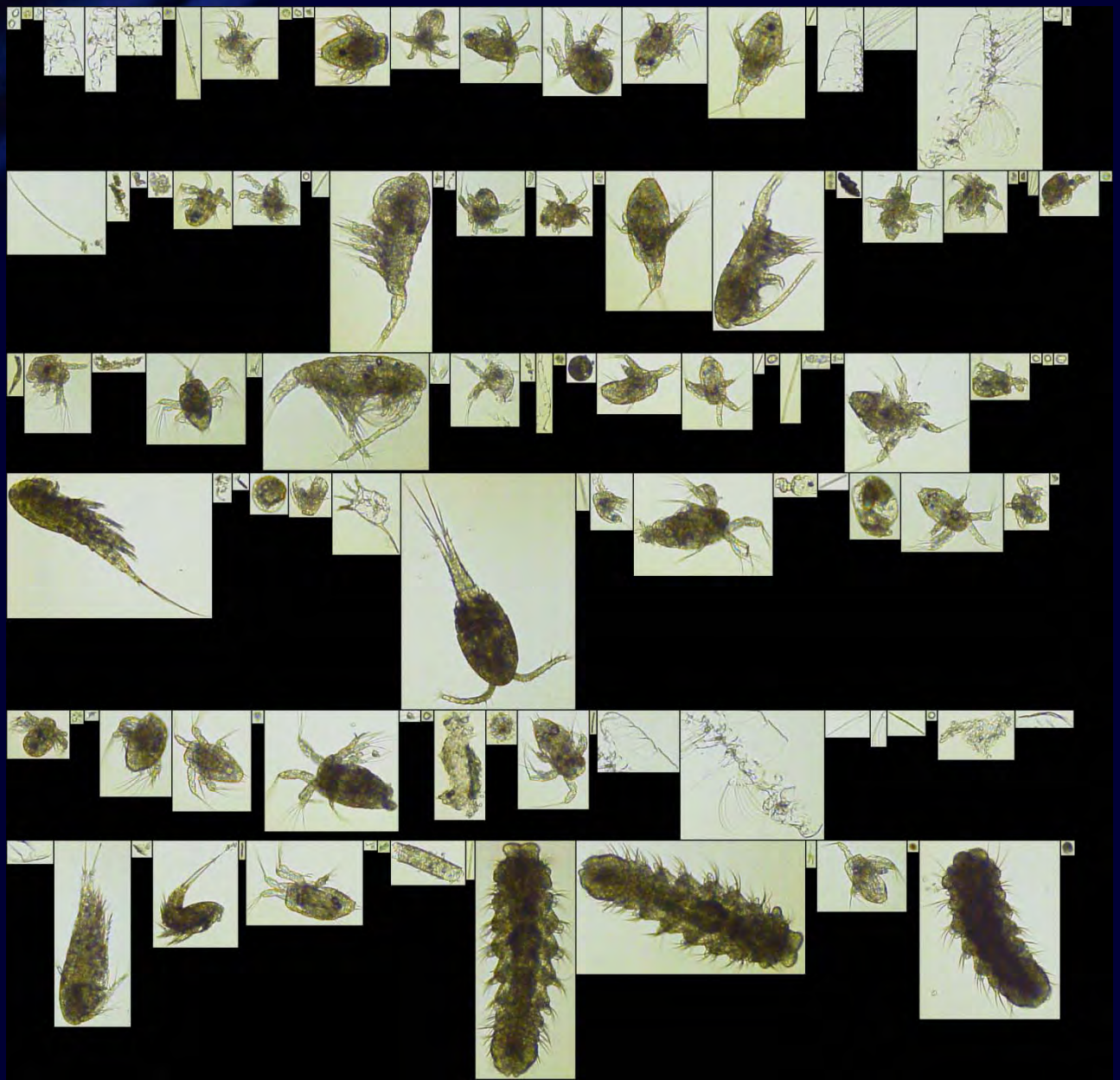
  Flow - .1 ml/min to 12 ml/min

  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

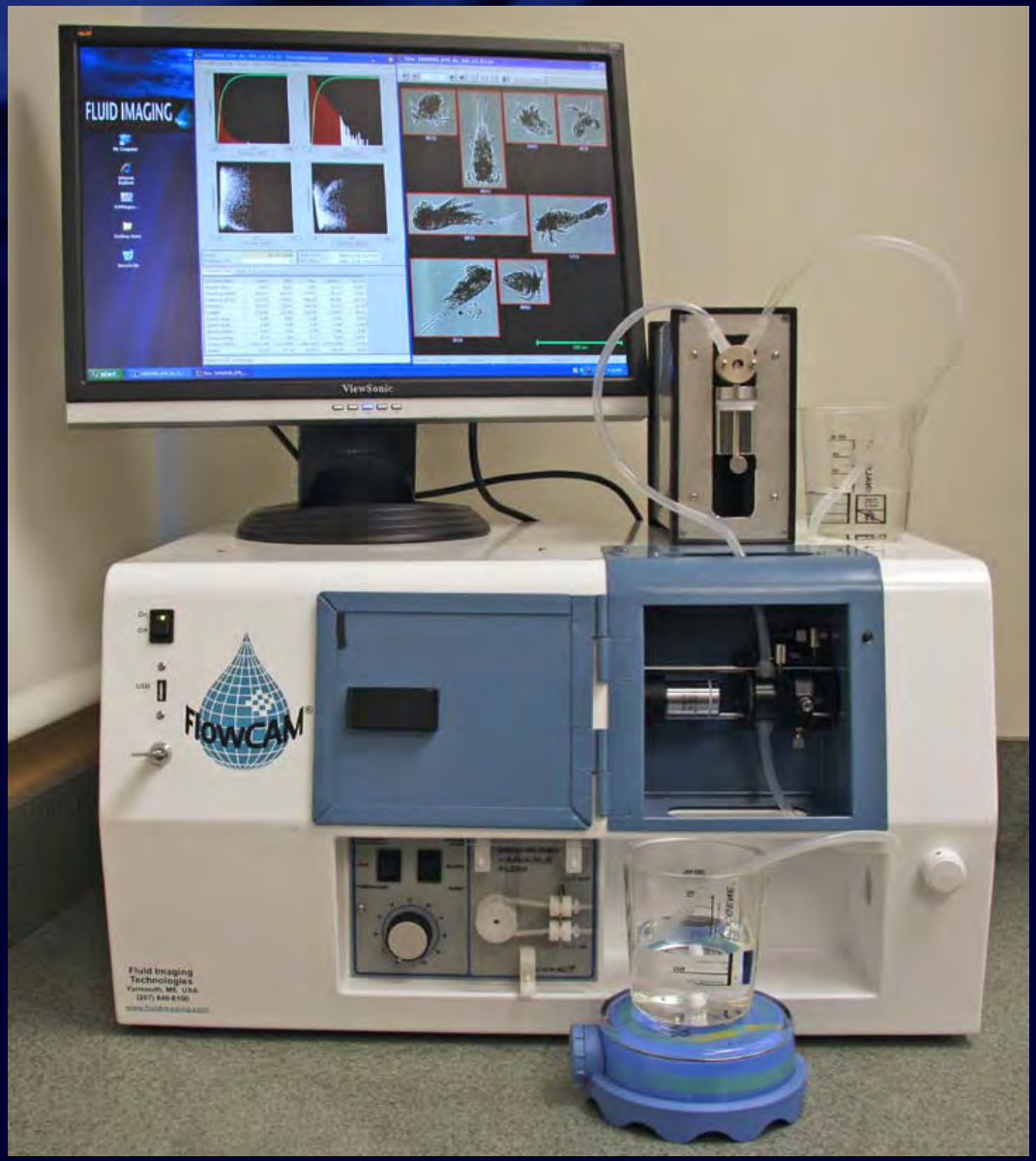
FlowCAM Applications (Industrial)

- 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

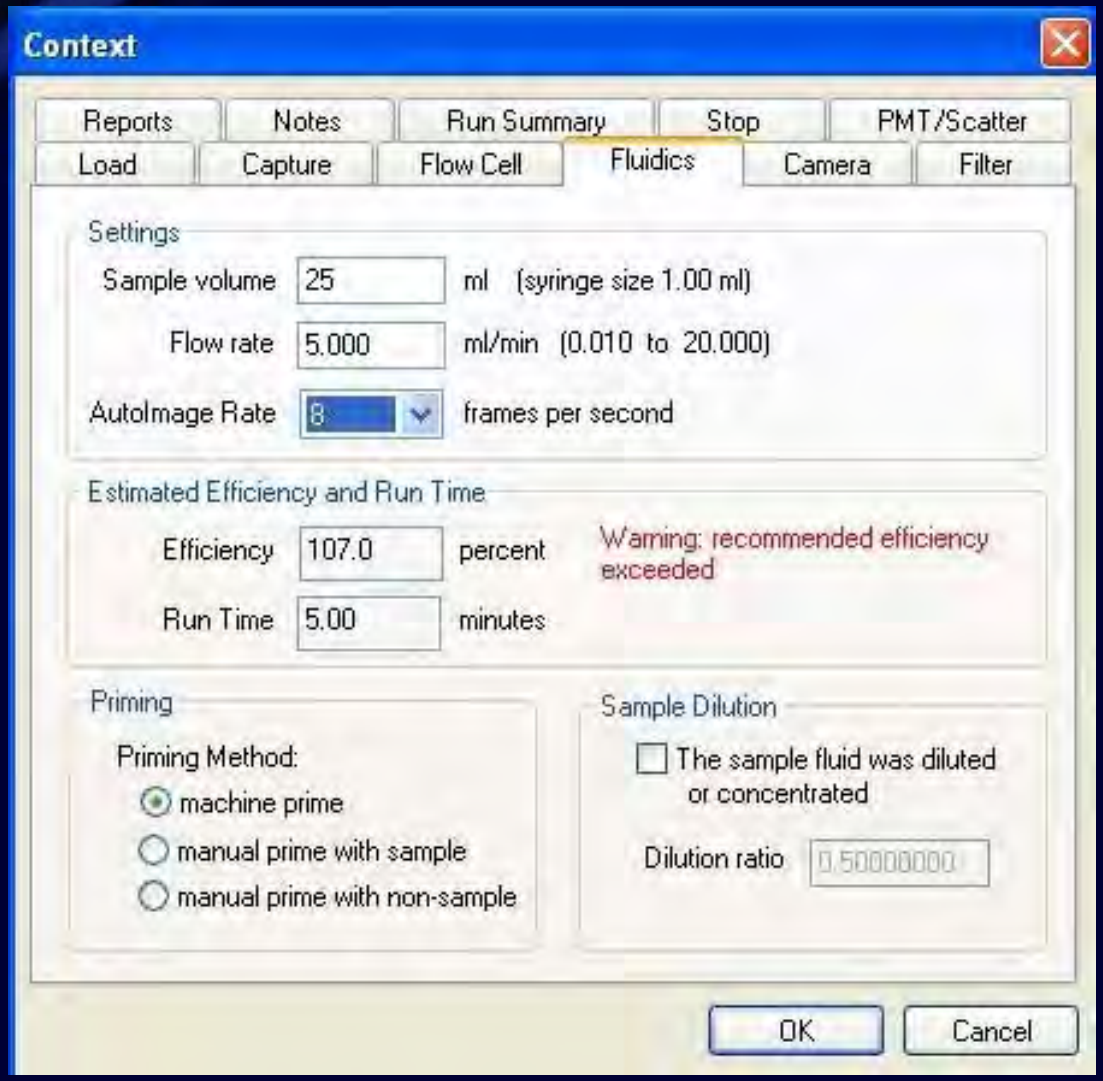
- Large particles settle quickly
- Large particles flow outside camera FOV
- Agglomeration of particles
  (esp. fixed samples)
- Camera view area (4.2 mm x 3.6 mm)
- Two dimensional image/Particle orientation

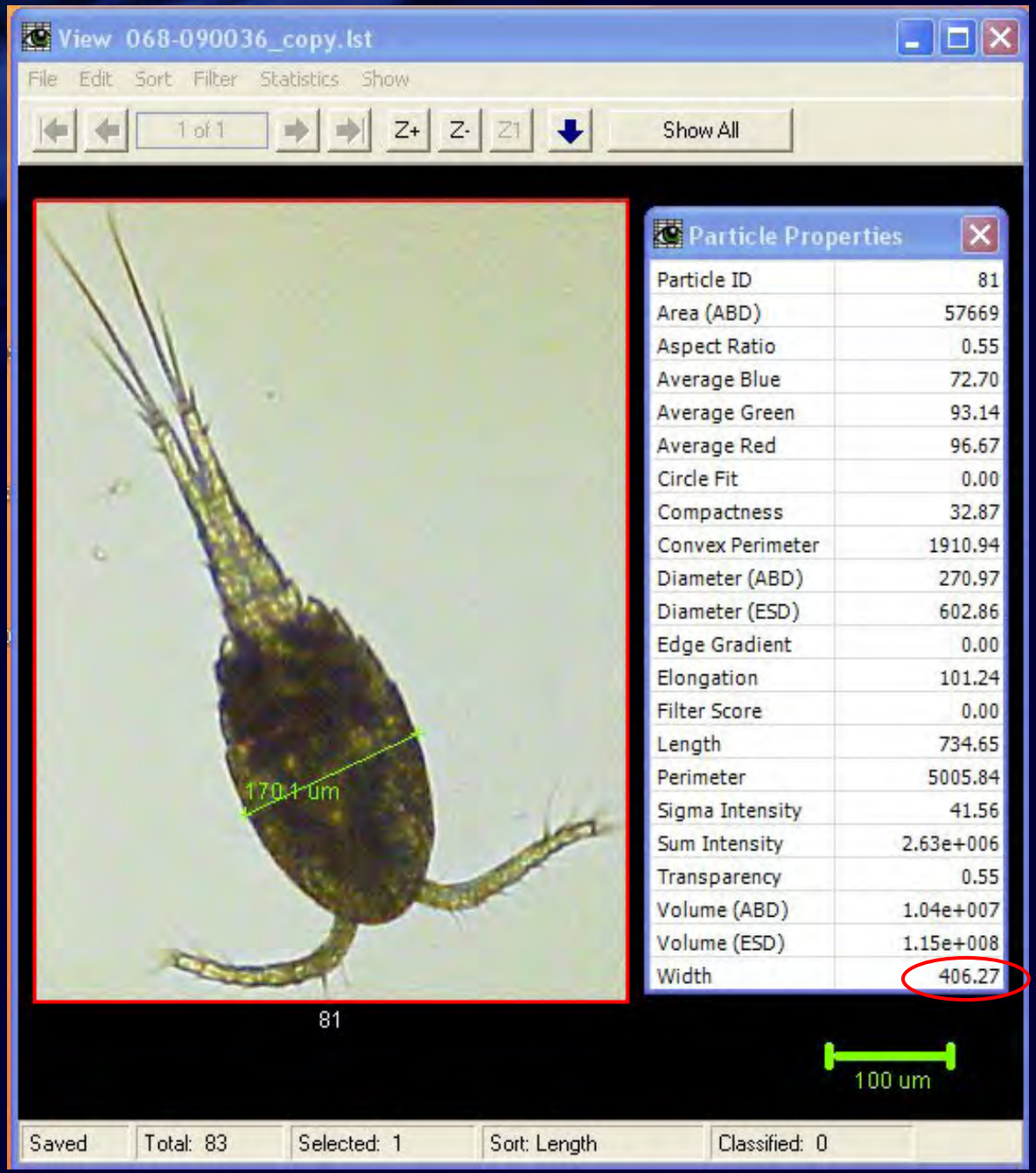
Zooplankton Set-up



- 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

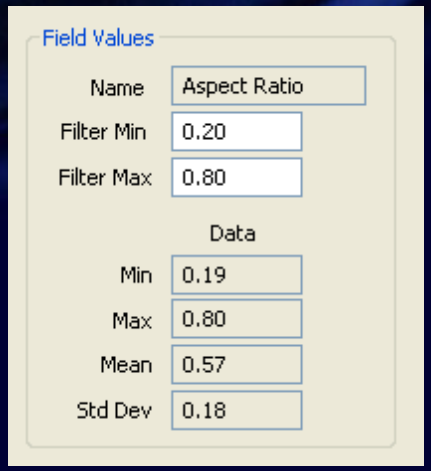




33 Image Parameters

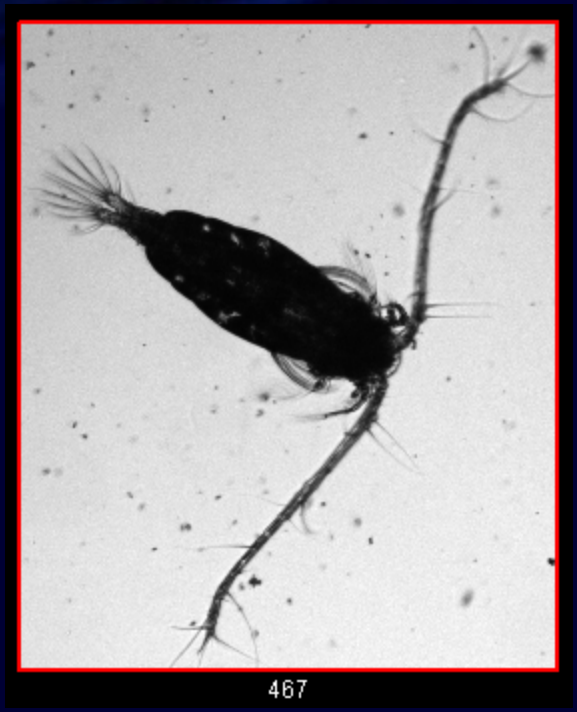
VisualSpreadsheet

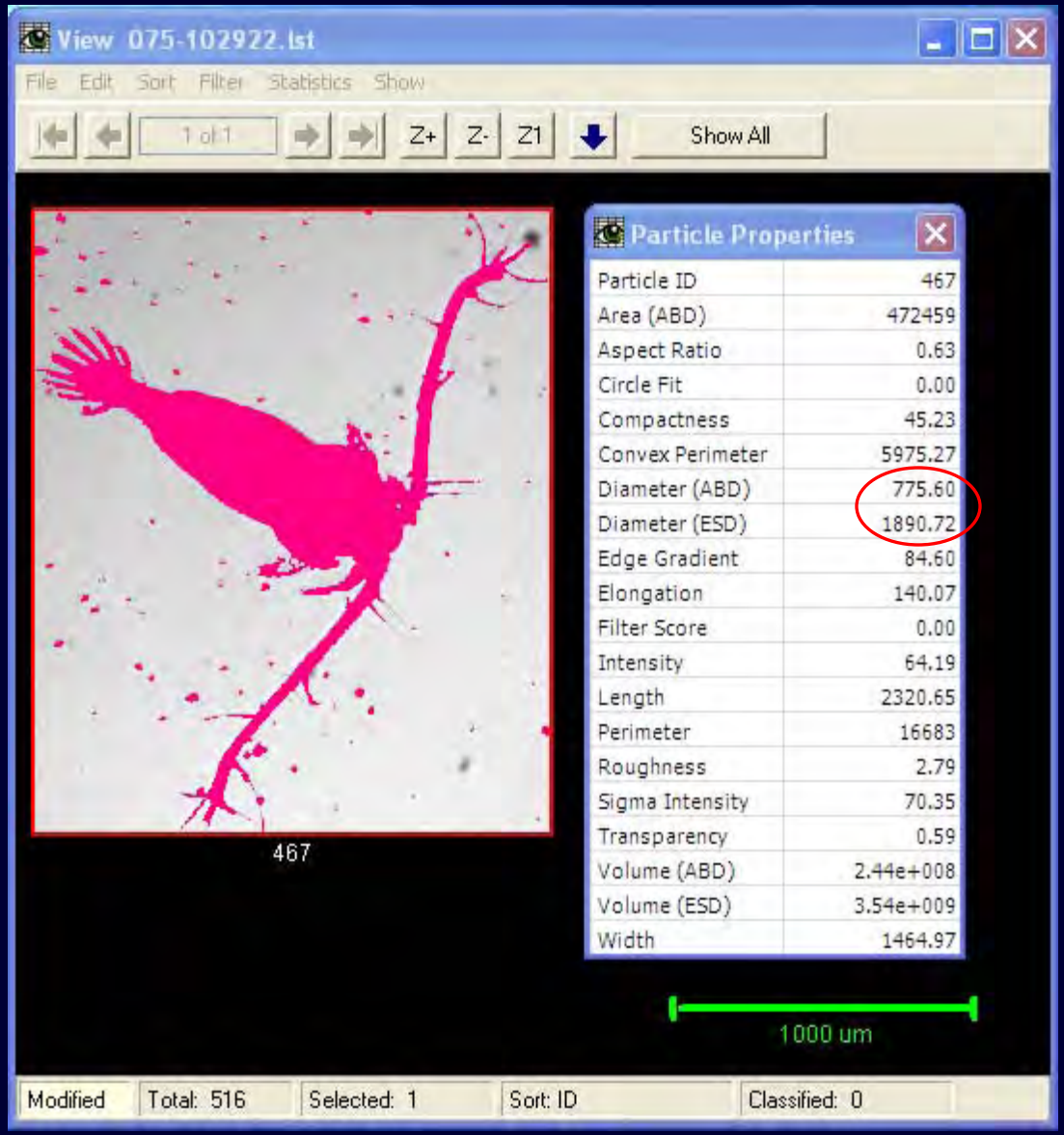
Automated Recognition Algorithm



- 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

Binary Image

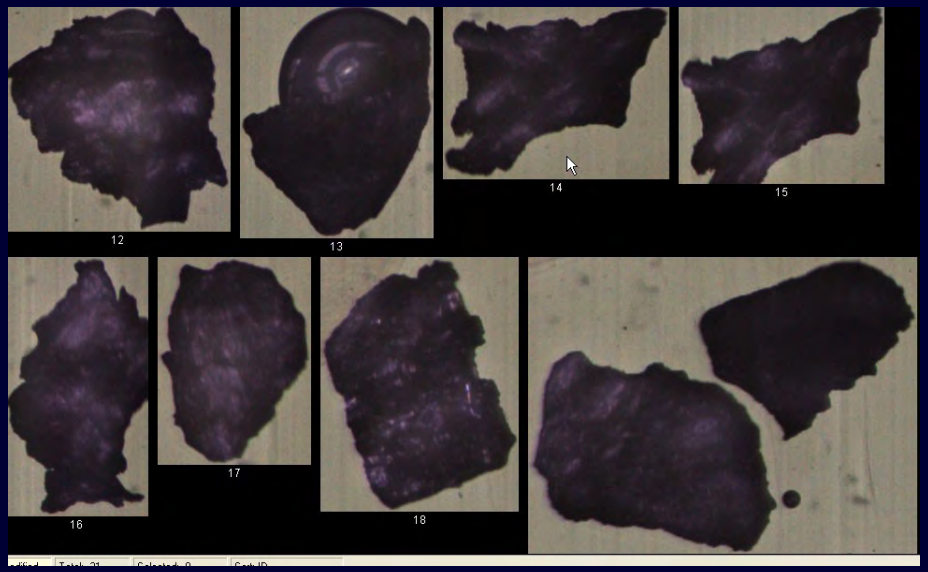




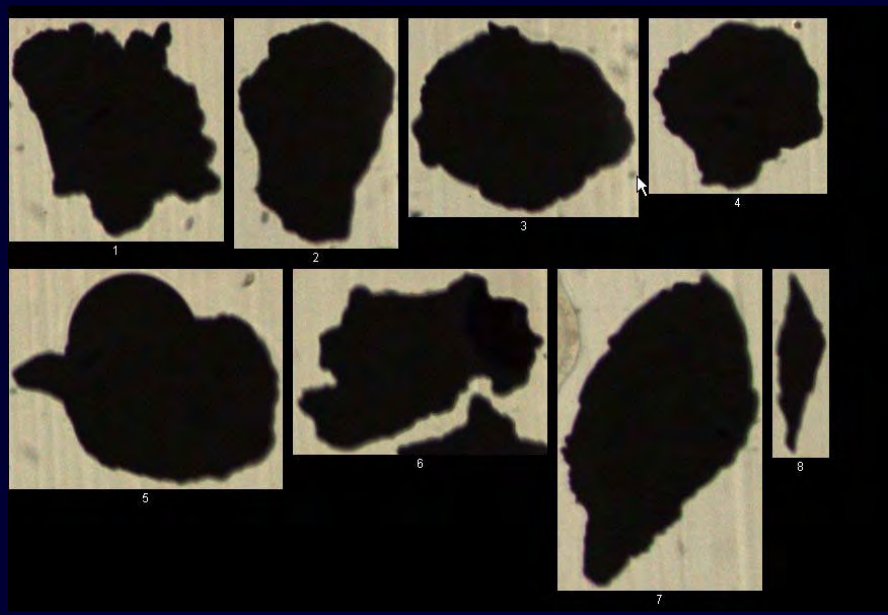
FlowCAM

Moving Forward

- ½ X Magnification
- Front Light LED Array



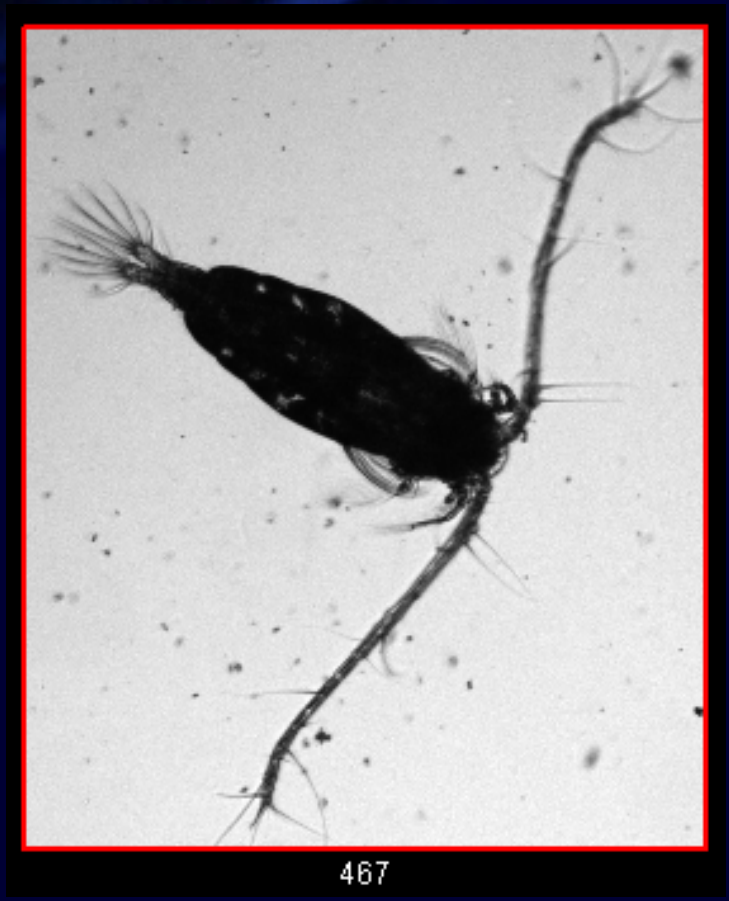
Front Light



Back Light

FlowCAM

Moving Forward



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:

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Matt Duplisea – Manager of Engineering
Ben Spaulding – Laboratory Manager
Bob Grimm – Senior Electronics Engineer