A Novel, Portable Flow Cytometer Facilitates Algal Population Quantification in Cultures and Environmental Samples

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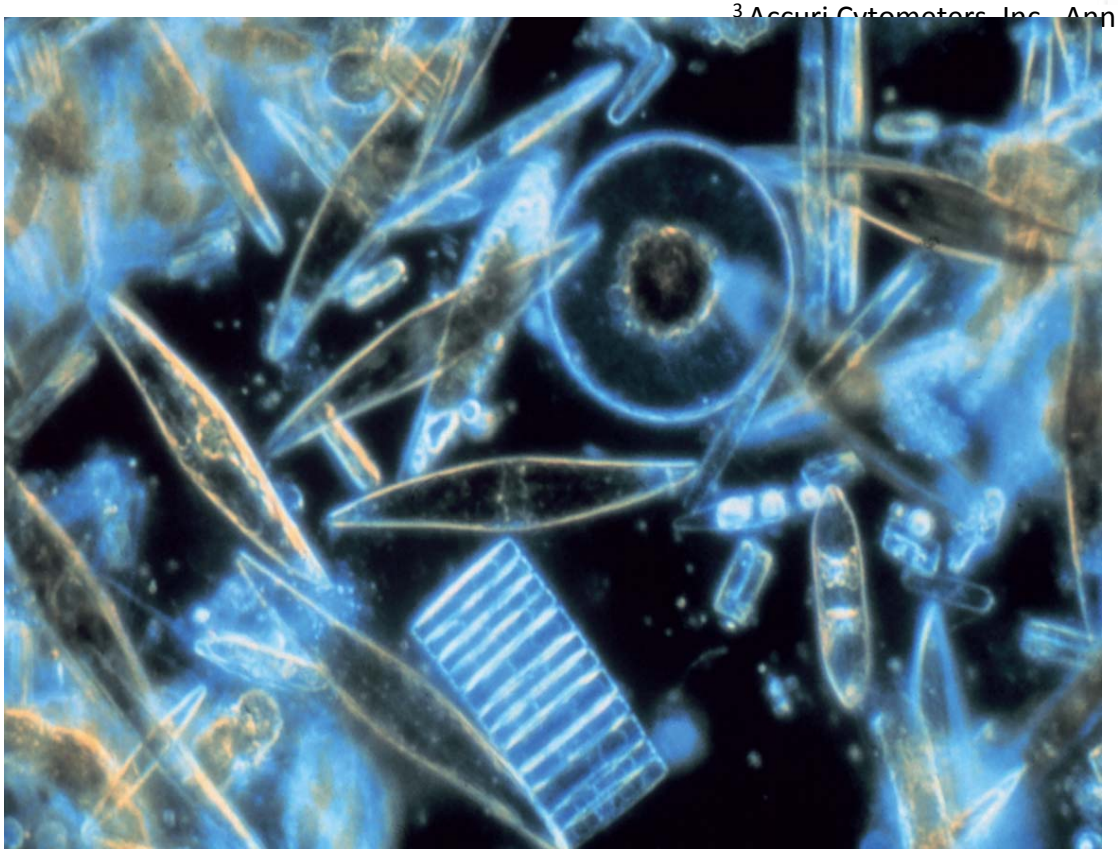
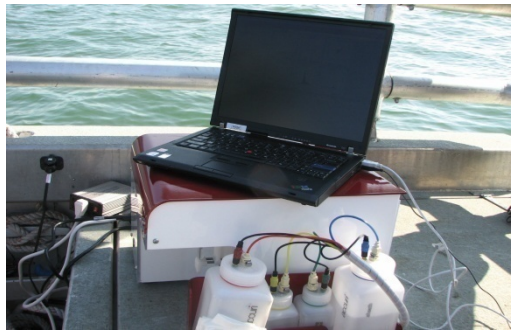




Small, Robust Flow Cytometer with Optional Automation

C6 with CSampler™

- 96-well plates
- 48-well plates
- 24-well plates
- 24-tube racks
- Etc.

C6 Flow Cytometer®

Flow Cytometer Evolution

Achieve equal results through innovation for less $

FACSCalibur: 90's state of the art
240 lbs, 36" x 24" x 26", Analog data

Accuri C6: 2010 replacement
25 lbs, 11" x 14" x 16", Digital data

Accuri C6- Standard Configuration

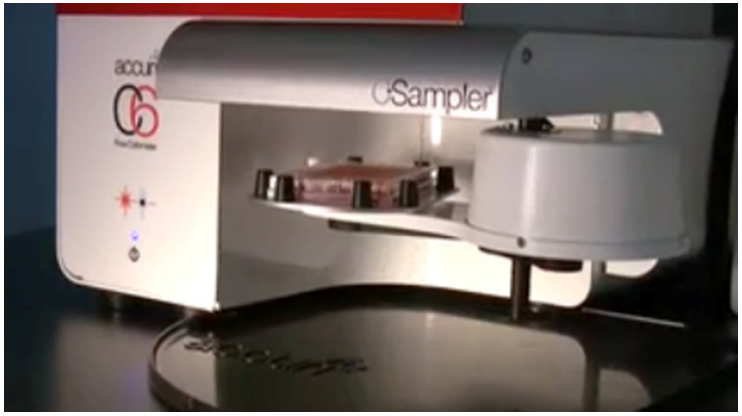
- 2 Lasers: 488nm-Blue, 640nm-Red
- 4 color detection:
  - FL1: FITC, GFP, Alexa488
  - FL2: PE, PI
  - FL3: PE-Cy5, PE-Cy7,PerCP, PerCp-Cy5.5, 7-AAD
  - FL4: APC, Alexa647
- Forward and Side Scatter detectors
- > 0.5 um particle size detection capabilities
- 10,000 event/sec collection rate

Accuri Innovations in Flow Cytometry

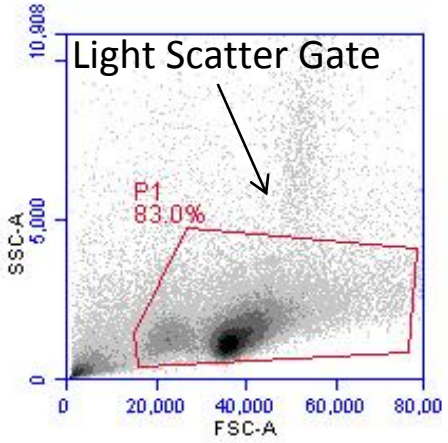
- **Fluidics**: allows direct-volume measurement
- **Optics:** locked-down alignment
- **Signal registration:** large dynamic range obviates voltage adjustments
- **Software:** developed by “high tech anthropologists” trained to facilitate human-computer interactions

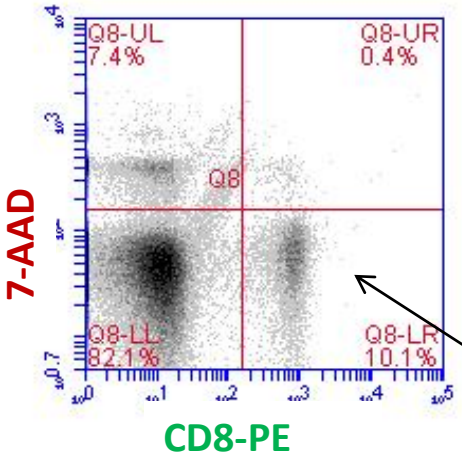
Unique Fluidics System Simplifies Sample Handling

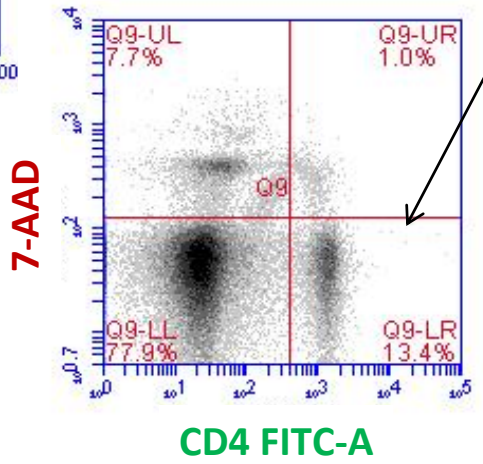
- Non-pressurized system
- Microprocessor-controlled peristaltic pumps enable direct volume measurement
- Many types of sample tubes may be used
- No need to transfer samples
- 30 ul sample volume
- Add reagents or cells during a run
- With CSampler: culture, stain and run, all in one plate



Calculation of Cells per Sample Volume





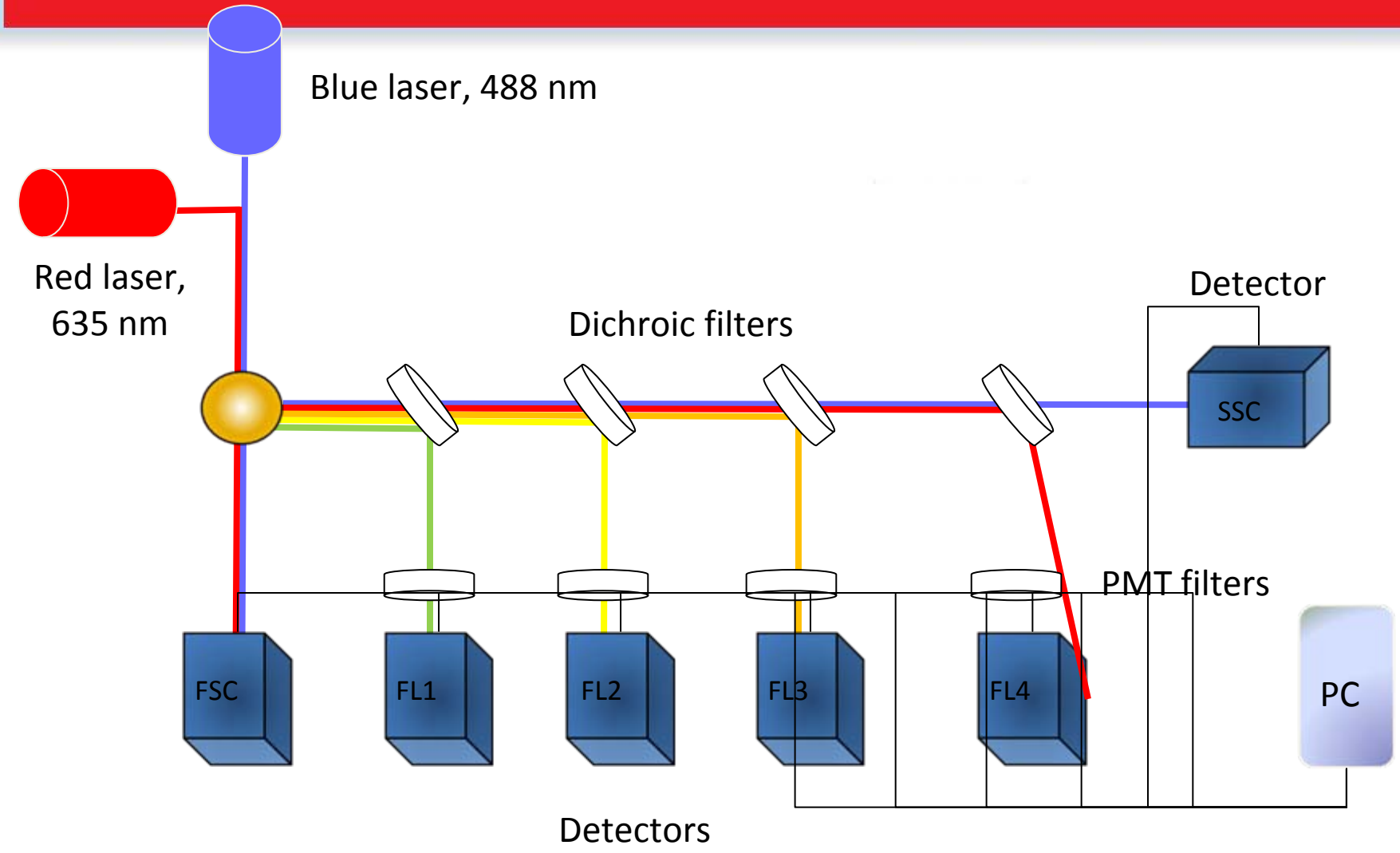


Plot 8: Sample A6 Gated on (P1 in all)	Count	Volume (µL)	Cells/µL	Phenotype
This Plot	58,588	6.4	9154	Total Cells in P1 Gate
Q8-UL	4,354	6.4		
Q8-UR	243	6.4		
Q8-LL	48,088	6.4		
Q8-LR	5,903	6.4	922	Viable CD8⁺ Cells

Viable T Cell Gates: Lower Right Quadrant

Plot 6: Sample A6 Gated on (P1 in all)	Count	Volume (µL)	Cells/µL	Phenotype
This Plot	58,588	6.4	9154	Total Cells in P1 Gate
Q9-UL	4,495	6.4		
Q9-UR	571	6.4		
Q9-LL	45,647	6.4		
Q9-LR	7,875	6.4	1230	Viable CD4⁺ Cells

Typical optical layout, 2 laser instruments



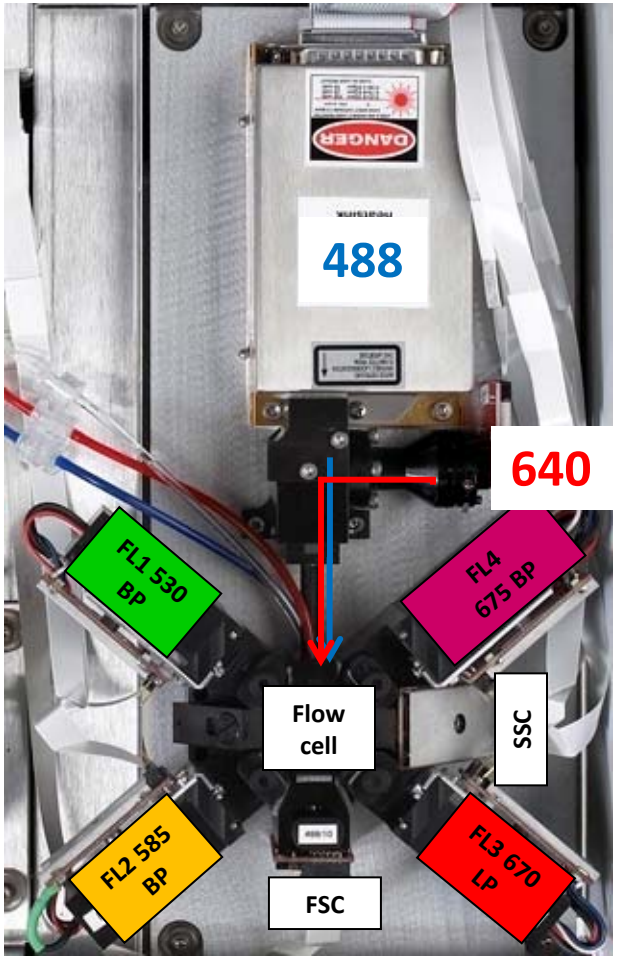
Accuri C6 Manufacturing Process:
Optical Alignment Optimized and Locked-down

Compact optical system design reduces cost and eliminates alignment issues

Cylindrical flow cell Allows PMTs to be situated at any angle

User changeable optical filters

510/15
540/20
565/20
610/20
780/60



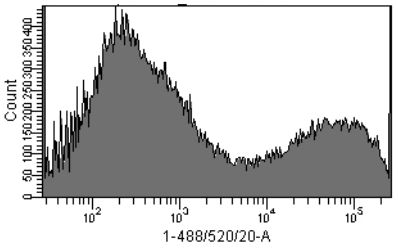
488 nm solid state laser

640 nm diode laser

Diodes for scatter detection

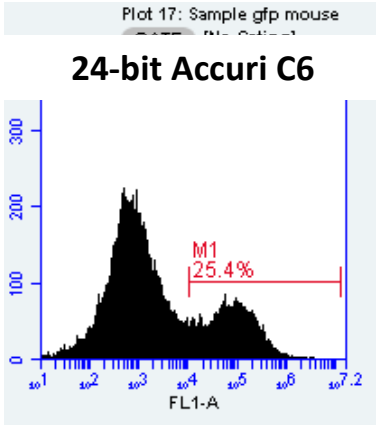
Signal Registration: Large Dynamic Range
24-bit Digital Signal Processing

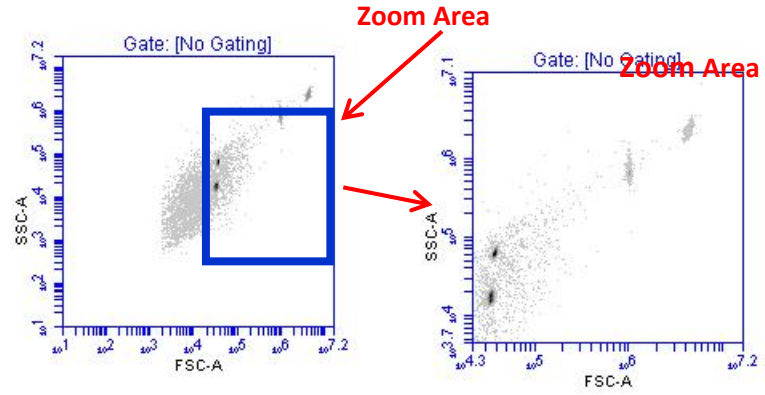
18-Bit System



"A GFP signal that was off scale on our other flow cytometer gave us a distribution that was contained within the 24-bit scale on the C6."

Ian Dimmick, Flow Cytometry Core Facility Manager, Institute of Human Genetics
Newcastle University, UK





Beads: 1, 2, 12, 29 micron

Dynamic Range:
7.2 decades of (log scale)
16,7 million (linear scale)

CFlow Software: Intuitive and User Friendly

Sample Grid

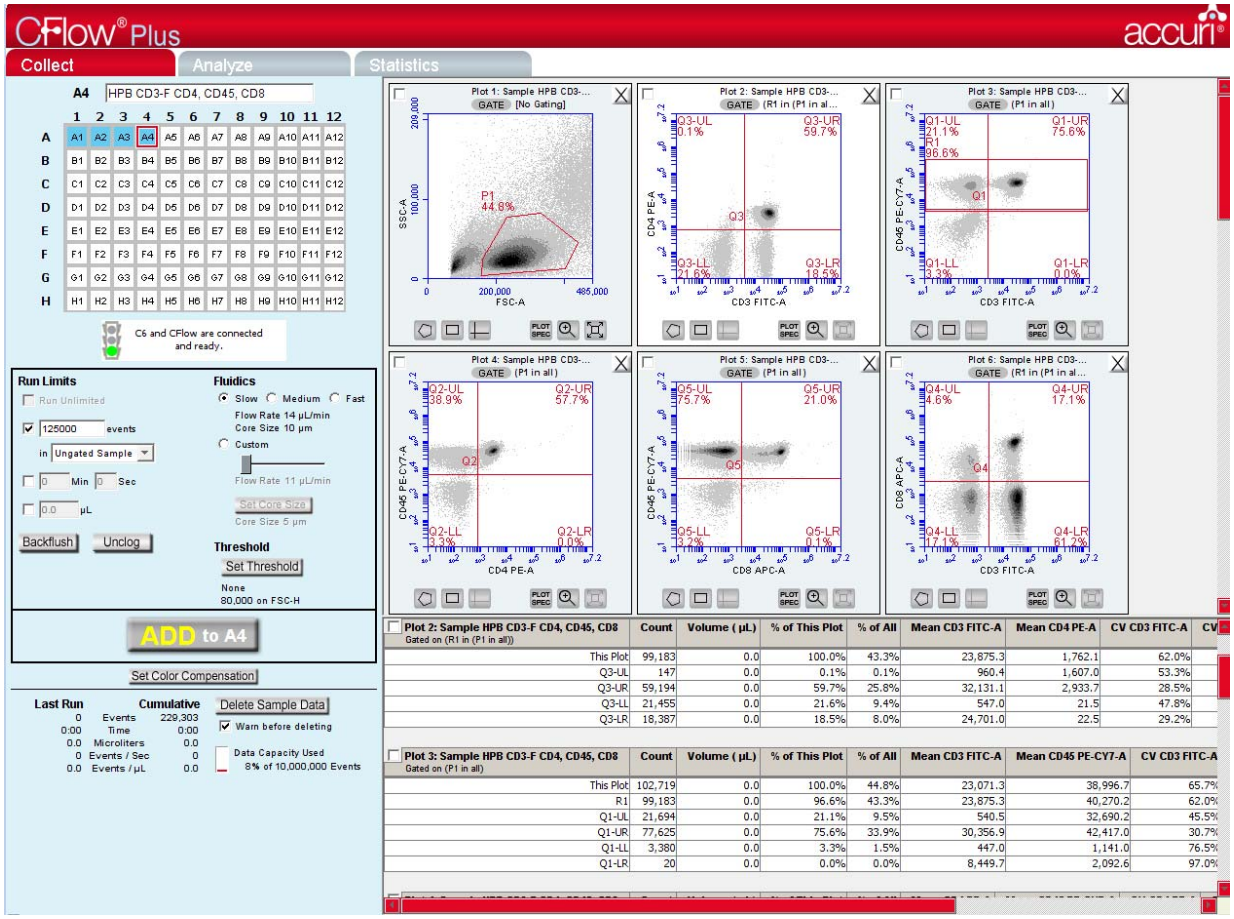
Cytometer Status

Fluidics Controls

Run Criteria

Threshold and Compensation

Real Time Updates



Analysis and Gating Tools

Regions

Quads

Markers

Histograms

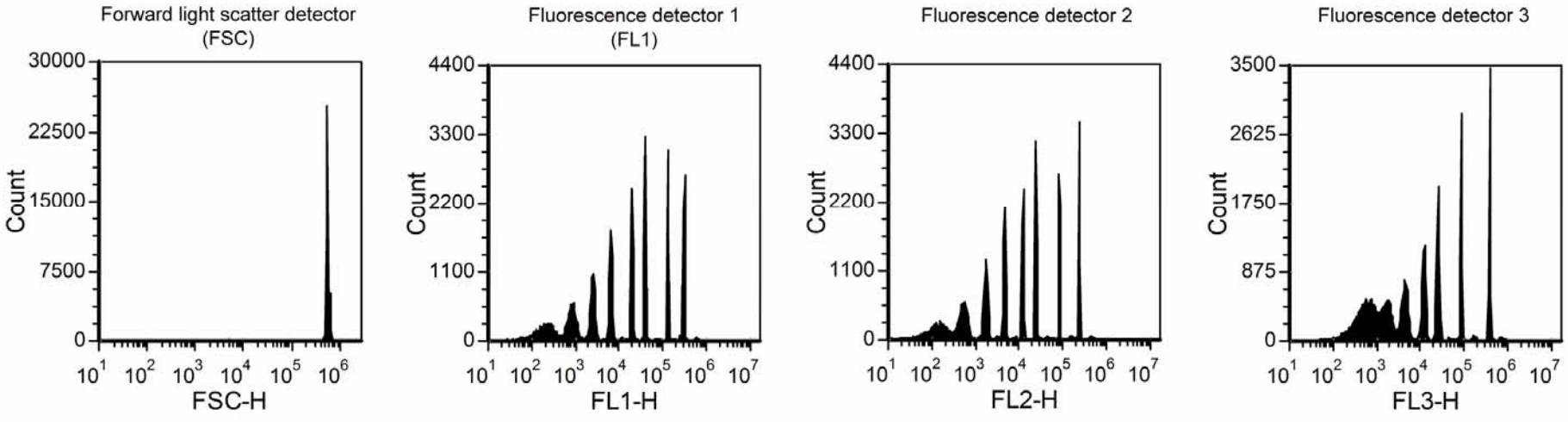
Dot plots

Density plots

Plot Statistics

Robust Locked-Down Optics, Simple On Site Validation

- **Pre-optimized, fixed voltages, locked-down optics at manufacture.**
- **No adjustments/alignments at setup.**
- **Performance validation using Spherotec Rainbow fluorescent beads.**

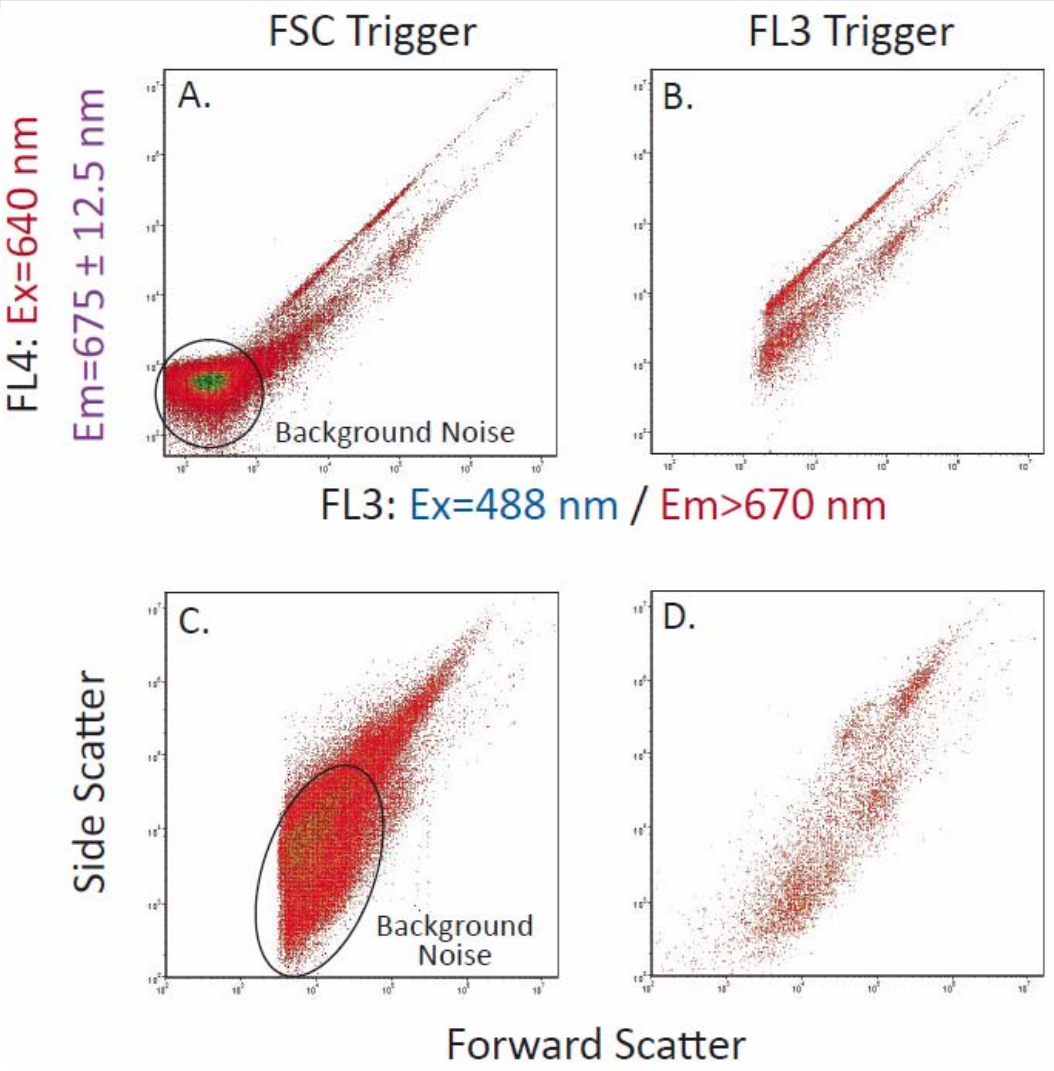


Intrinsic Fluorochromes Detected by the Accuri C6

Fluorophore	Exciting Laser	Major Emission Wavelength	C6 Detector (filter)
Chlorophyll *a,b*	488	>640 nm	FL3 (670 LP)
Phycoerythrin	488	575 nm	FL2 (585 ± 20)
C-phycocyanin	640	650 nm	FL4 (675 ± 12.5)
R-phycocyanin	640	646 nm	FL4 (675 ± 12.5)
Allophycocyanin	640	660 nm	FL4 (675 ± 12.5)

Naturally-occurring fluorescent pigments in phytoplankton and the C6 detector where major fluorescence signal for each is expected.

Data Collection: Triggering on Forward Scatter vs. Fluorescence

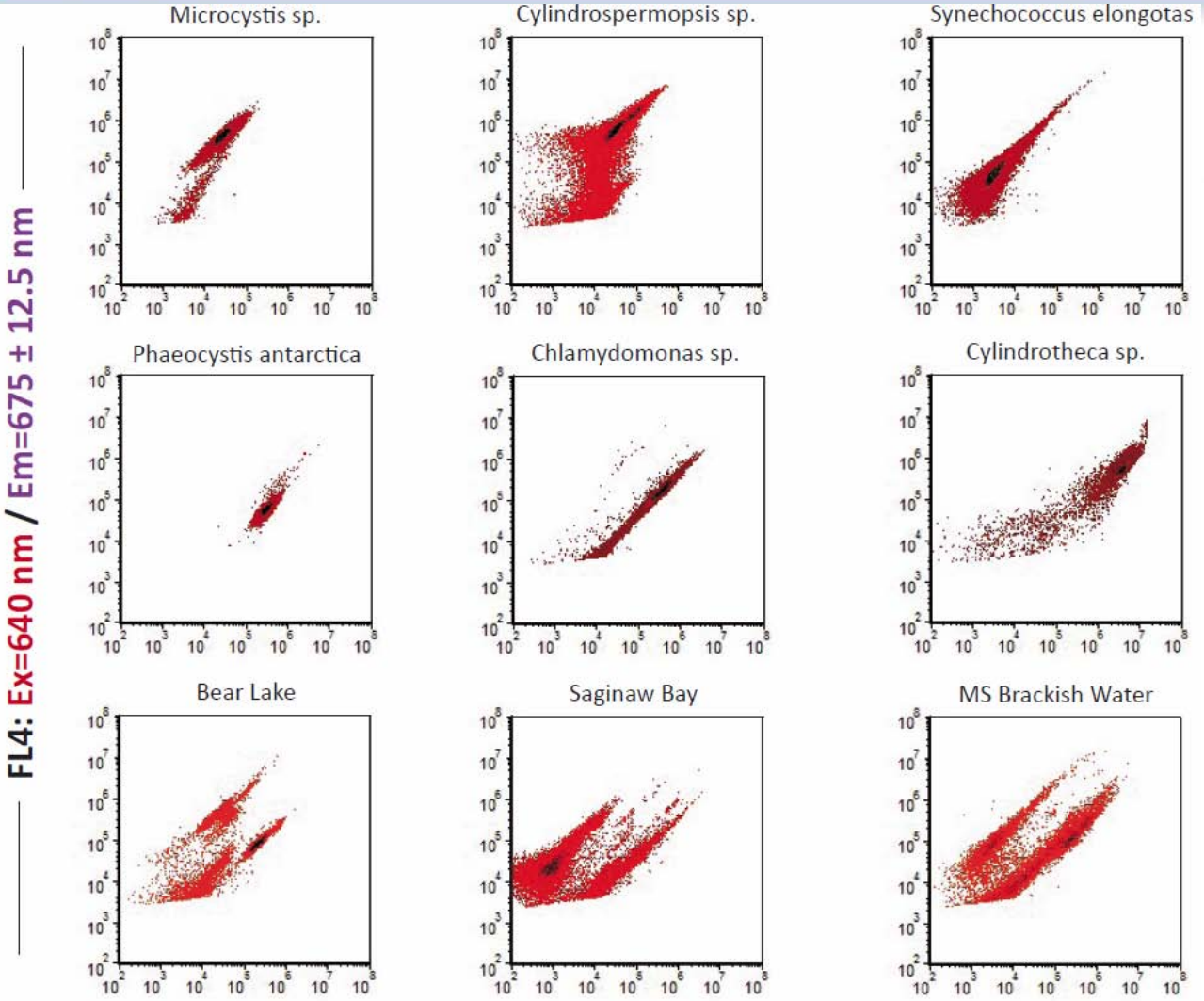


Surface water samples collected from Lake Erie.

A and **C**: acquired using a forward scatter trigger.

B and **D**: acquired triggering on chlorophyll fluorescence (FL3)

Examples of Intrinsic Fluorescence

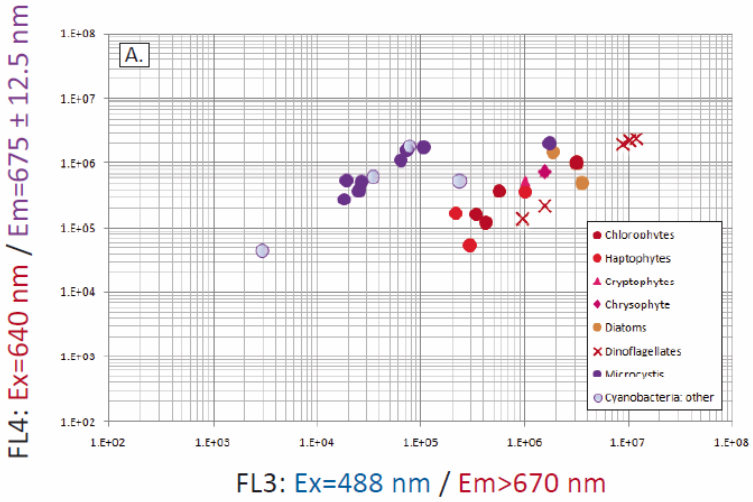


Phycocyanin fluorescence (FL4) dominates

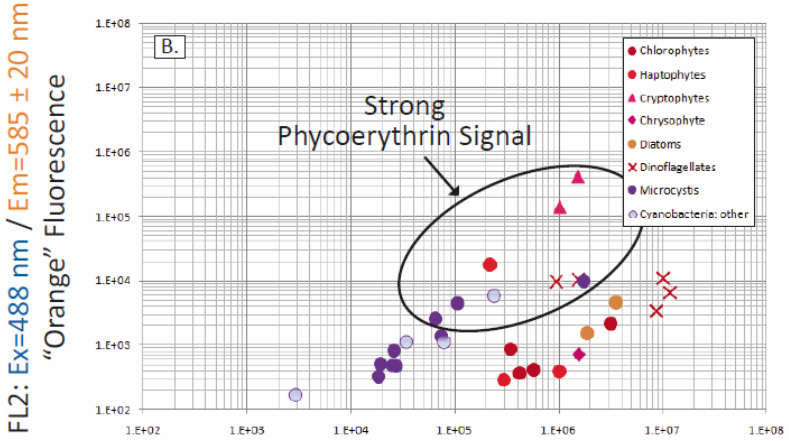
Chlorophyll accessory pigment fluorescence (FL3) dominates

Environmental samples with mixed FL3- and FL4-fluorescent cells

Compiled Fluorescence Data: Algal Cultures

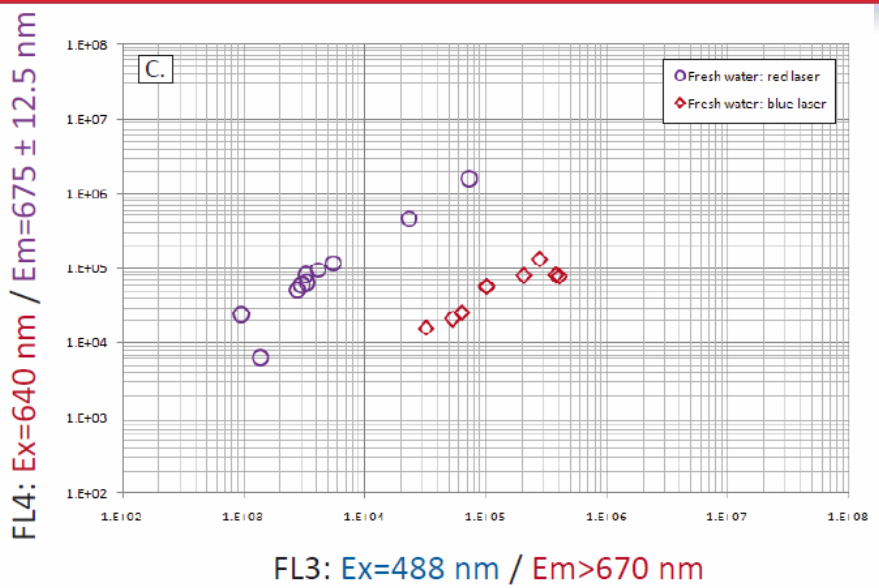


Grouping based on red fluorescence signals: with 488 nm excitation (chlorophyll, FL3) and with 640 nm excitation (phycocyanin, FL4)

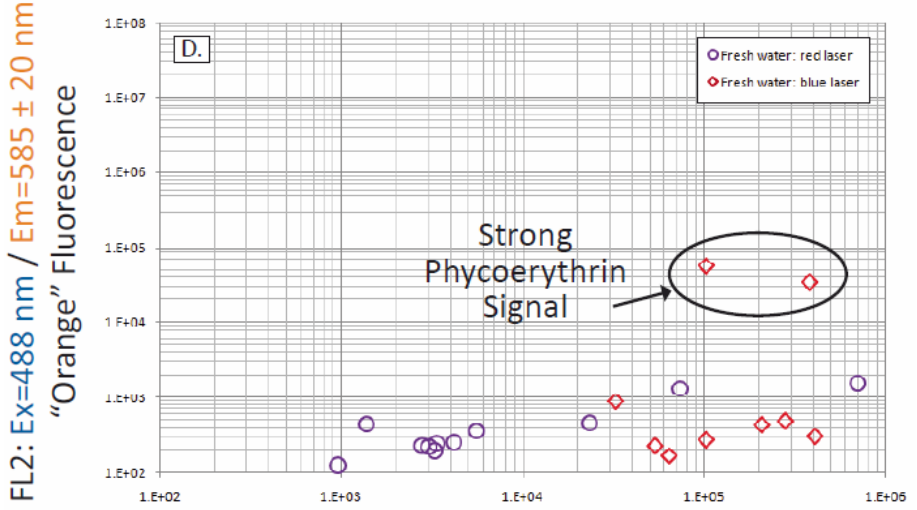


Grouping based on "orange" phycoerythrin fluorescence (FL2) and red chlorophyll fluorescence (FL3)

Compiled Fluorescence Data: Fresh Water Samples



Grouping based on two red fluorescence signals: with 488 nm excitation (chlorophyll, FL3) and with 640 nm excitation (phycocyanin, FL4)



Grouping based on “orange” phycoerythrin fluorescence (FL2) and red chlorophyll fluorescence (FL3)

Cell Counts of 3 Fluorescence Signature-Defined Populations in Fresh Water Samples

Source	Site	Population Concentration (per mL)		
		phycocyanin	chlorophyll a,b*	phycoerythrin*
Saginaw Bay (MI)	1	19,075	19,120	0
Saginaw Bay (MI)	2	20,045	53,275	0
Saginaw Bay (MI)**	4	3,570	17,255	11,180
Saginaw Bay (MI)	23	3,080	5,975	0
Saginaw Bay (MI)**	BCW-B	2,993	22,207	8,807
Saginaw Bay (MI)	SB5-S	3,307	30,233	0
Bear Lake (MI)		12,640	23,240	0
Lake Mead (NE)		33,462	8,216	0
Waughop Lake (WA)		6,137	9,788	0
Brackish water (MI)		450,563	154,354	0

Algal Cultures Analyzed with the C6 Flow Cytometer

Cyanobacteria
Microcystis aeruginosa
Cylindrospermopsis racibor
Synechococcus elongatus
Anabaena flos-aquae
Aphanizomen flos-aquae

Chlorophytes (Green Algae)
Chlamydomonas sp.
Selenastrum sp.
Chlorella sp.
Tetraedron sp.
Tetraselmis suecica

Haptophytes
Chrysochromulina sp.
Phaeocystis antarctica
Prymnesium sp.

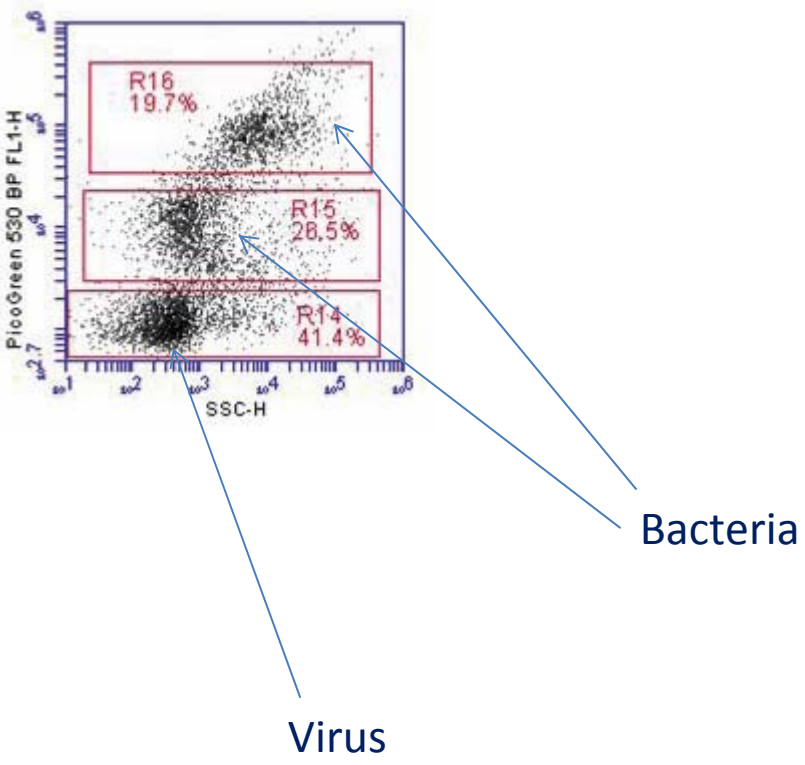
Cryptophytes
Rhodomonas salina

Chrysophyte
Chromulina

Diatoms
Pediastrum simplex
Cylindrotheca sp.

Dinoflagellates
Alexandrium sp.
Prorocentrum sp.
Karenia sp.

Heterotrophic Bacteria and Viruses



Detection of aquatic bacteria (R15 and R16) and virus (R14) using a nucleic acid stain (PicoGreen, Invitrogen), a fluorescent dye that selectively binds dsDNA, and side scatter (SSC). Data provided courtesy of Marcel Veldhuis, PhD, Royal Netherlands Institute for Sea Research (NIOZ).

Installed Customer Base and Support

Major Customer Institutions

- UC System
  - UCSF, UCSD, UCLA, UCR, UCI, UCD, UCSB
- Stanford
- Harvard
- MIT
- NIH
- Mayo Clinic
- U of WA
- UT Austin
- U of UT
- Washington University
- Cleveland Clinic
- U of MI
- Emory University
- Duke University
- University of Wisconsin
- University of Rochester
- University of Pittsburgh
- HHMI
- CalTech

- More than 500 systems installed since 2008
- Global headquarters in US
- European office in UK
- Field specialists in US and Europe

Designed and Manufactured in Ann Arbor, MI, U.S.A.

Accuri Cytometers, Inc. manufacturing facility in Ann Arbor, MI.
Accuri C6 Flow Cytometer complies with the **Buy American Act.**