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 $
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

**Viable T Cell Gates: Lower Right Quadrant**

### Plot 8: Sample A6

<table>
<thead>
<tr>
<th>Count</th>
<th>Volume (μL)</th>
<th>Cells/μL</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total Cells in P1 Gate</td>
</tr>
<tr>
<td>This Plot</td>
<td>58,588</td>
<td>6.4</td>
<td>9154</td>
</tr>
<tr>
<td>Q8-UL</td>
<td>4,354</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>Q8-UR</td>
<td>243</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>Q8-LL</td>
<td>48,088</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>Q8-LR</td>
<td>5,903</td>
<td>6.4</td>
<td>922 Viable CD8+ Cells</td>
</tr>
</tbody>
</table>

### Plot 6: Sample A6

<table>
<thead>
<tr>
<th>Count</th>
<th>Volume (μL)</th>
<th>Cells/μL</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total Cells in P1 Gate</td>
</tr>
<tr>
<td>This Plot</td>
<td>58,588</td>
<td>6.4</td>
<td>9154</td>
</tr>
<tr>
<td>Q9-UL</td>
<td>4,495</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>Q9-UR</td>
<td>571</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>Q9-LL</td>
<td>45,647</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>Q9-LR</td>
<td>7,875</td>
<td>6.4</td>
<td>1230 Viable CD4+ Cells</td>
</tr>
</tbody>
</table>
Typical optical layout, 2 laser instruments

Blue laser, 488 nm

Red laser, 635 nm

Dichroic filters

PMT filters

Detectors

SSC

Detector

PC
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
“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

- Analysis and Gating Tools
- Regions
- Quads
- Markers
- Histograms
- Dot plots
- Density plots
- Plot Statistics

Sample Grid
Cytometer Status
Fluidics Controls
Run Criteria
Threshold and Compensation
Real Time Updates
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

<table>
<thead>
<tr>
<th>Fluorophore</th>
<th>Exciting Laser</th>
<th>Major Emission Wavelength</th>
<th>C6 Detector (filter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll (a, b)</td>
<td>488</td>
<td>&gt;640 nm</td>
<td>FL3 (670 LP)</td>
</tr>
<tr>
<td>Phycoerythrin</td>
<td>488</td>
<td>575 nm</td>
<td>FL2 (585 ± 20)</td>
</tr>
<tr>
<td>C-phycocyanin</td>
<td>640</td>
<td>650 nm</td>
<td>FL4 (675 ± 12.5)</td>
</tr>
<tr>
<td>R-phycocyanin</td>
<td>640</td>
<td>646 nm</td>
<td>FL4 (675 ± 12.5)</td>
</tr>
<tr>
<td>Allophycocyanin</td>
<td>640</td>
<td>660 nm</td>
<td>FL4 (675 ± 12.5)</td>
</tr>
</tbody>
</table>

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

FL4: Ex=640 nm / Em=675 ± 12.5 nm

FL3: Ex=488 nm / Em>670 nm
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

<table>
<thead>
<tr>
<th>Source</th>
<th>Site</th>
<th>Population Concentration (per mL)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>phycocyanin</td>
<td>chlorophyll a,b*</td>
<td>phycoerythrin*</td>
<td></td>
</tr>
<tr>
<td>Saginaw Bay (MI)</td>
<td>1</td>
<td>19,075</td>
<td>19,120</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Saginaw Bay (MI)</td>
<td>2</td>
<td>20,045</td>
<td>53,275</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Saginaw Bay (MI)**</td>
<td>4</td>
<td>3,570</td>
<td>17,255</td>
<td>11,180</td>
<td></td>
</tr>
<tr>
<td>Saginaw Bay (MI)</td>
<td>23</td>
<td>3,080</td>
<td>5,975</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Saginaw Bay (MI)**</td>
<td>BCW-B</td>
<td>2,993</td>
<td>22,207</td>
<td>8,807</td>
<td></td>
</tr>
<tr>
<td>Saginaw Bay (MI)</td>
<td>SB5-S</td>
<td>3,307</td>
<td>30,233</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Bear Lake (MI)</td>
<td></td>
<td>12,640</td>
<td>23,240</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Lake Mead (NE)</td>
<td></td>
<td>33,462</td>
<td>8,216</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Waughop Lake (WA)</td>
<td></td>
<td>6,137</td>
<td>9,788</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Brackish water (MI)</td>
<td></td>
<td>450,563</td>
<td>154,354</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
### Algal Cultures Analyzed with the C6 Flow Cytometer

<table>
<thead>
<tr>
<th><strong>Cyanobacteria</strong></th>
<th><strong>Chlorophytes (Green Algae)</strong></th>
<th><strong>Haptophytes</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcystis aeruginosa</td>
<td>Chlamydomonas sp.</td>
<td>Chrysochromulina sp.</td>
</tr>
<tr>
<td>Cylindrospermopsis racibor</td>
<td>Selenastrum sp.</td>
<td>Phaeocystis antarctica</td>
</tr>
<tr>
<td>Synechococcus elongatus</td>
<td>Chlorella sp.</td>
<td>Prymnesium sp.</td>
</tr>
<tr>
<td>Anabaena flos-aquae</td>
<td>Tetraedron sp.</td>
<td></td>
</tr>
<tr>
<td>Aphanizomen flos-aquae</td>
<td>Tetraselmis suecica</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Cryptophytes</strong></th>
<th><strong>Chrysophyte</strong></th>
<th><strong>Diatoms</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhodomonas salina</td>
<td>Chromulina</td>
<td>Pediasstrum simplex</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Dinoflagellates</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alexandrium sp.</td>
</tr>
<tr>
<td>Prorocentrum sp.</td>
</tr>
<tr>
<td>Karenia sp.</td>
</tr>
</tbody>
</table>
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.**