The BD Accuri C6 Flow Cytometer ®

A powerful system with a simple design.

Charlie Hsu 許博彥

Instrument Product Specialist
騰達行/Unimed Healthcare INC.

BD

UNIMED 騰達行企業股份有限公司 UNIMED HEALTHCARE INC.

Accuri's Mission

Proliferate the
power of flow cytometry
to every lab that works with
cells, beads, or particles
worldwide.

Accuri is Enabling Discovery

Survey's top four reasons for choosing a flow cytometer match Accuri's key features

1. Ease of Use – 1 hour vs. 2-5 days to learn
2. Size – 1/6 the size of market leaders
3. Cost – 1/3 the cost of market leaders
4. Dyes (colors) - Accuri's specs meets the requirements of 92% of users

Percentage of Respondents Using Each FC Dye – 80% 4 colors or less

1st FITC	38%	4th GFP	20%	7th PerCP	14%
2nd PE	27%	5th Alexa 488	16%	8th APC	13%
3rd PI	26%	6th Cy5	15%		

All dyes above can be run on the standard C6.

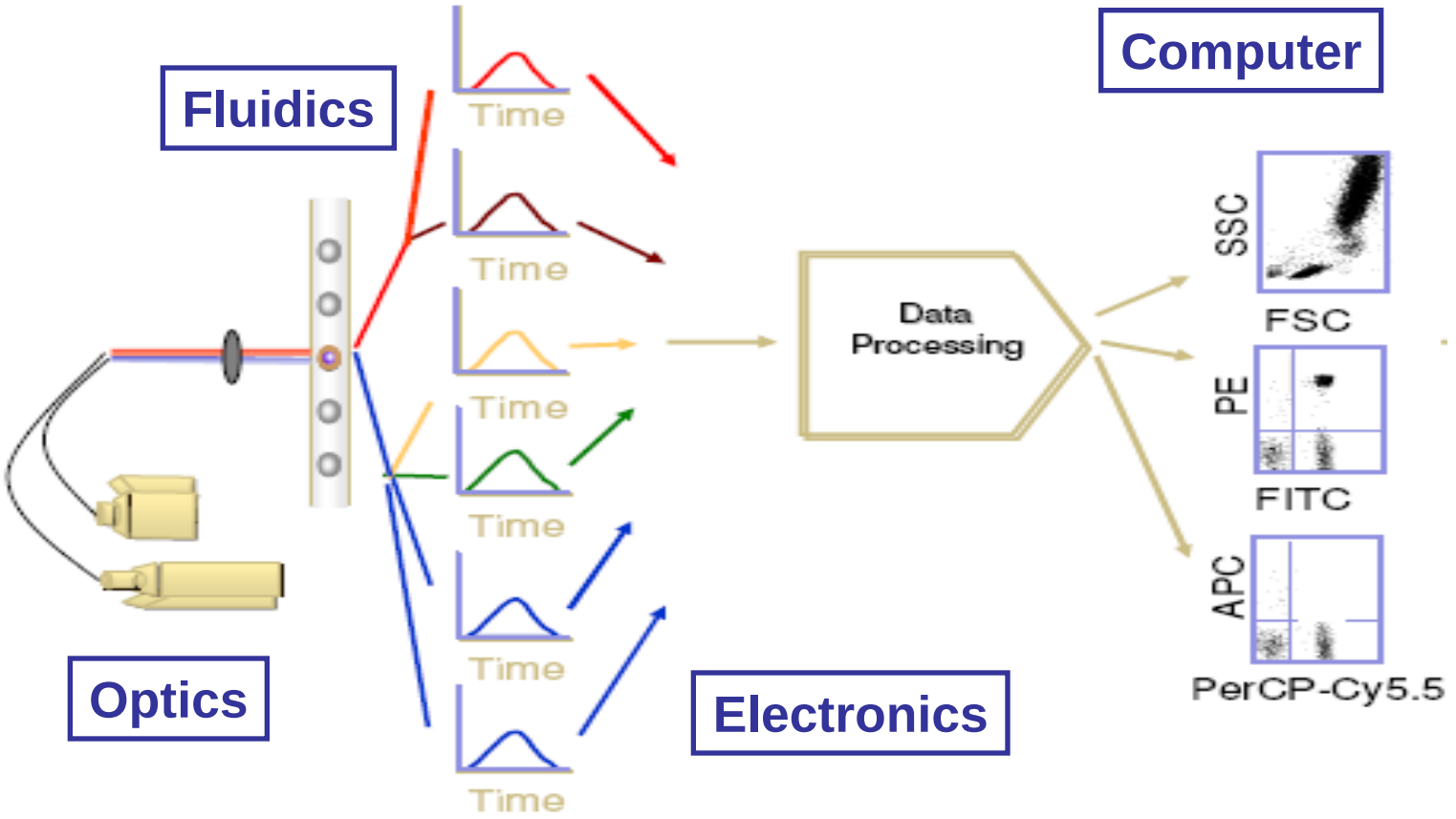
Source: 8/2008 Biocompare Survey of Flow Cytometry Users

The C6 Flow Cytometer

Innovations in all the major components of a flow cytometer

- Fluidics
- Optics
- Electronics
- Software

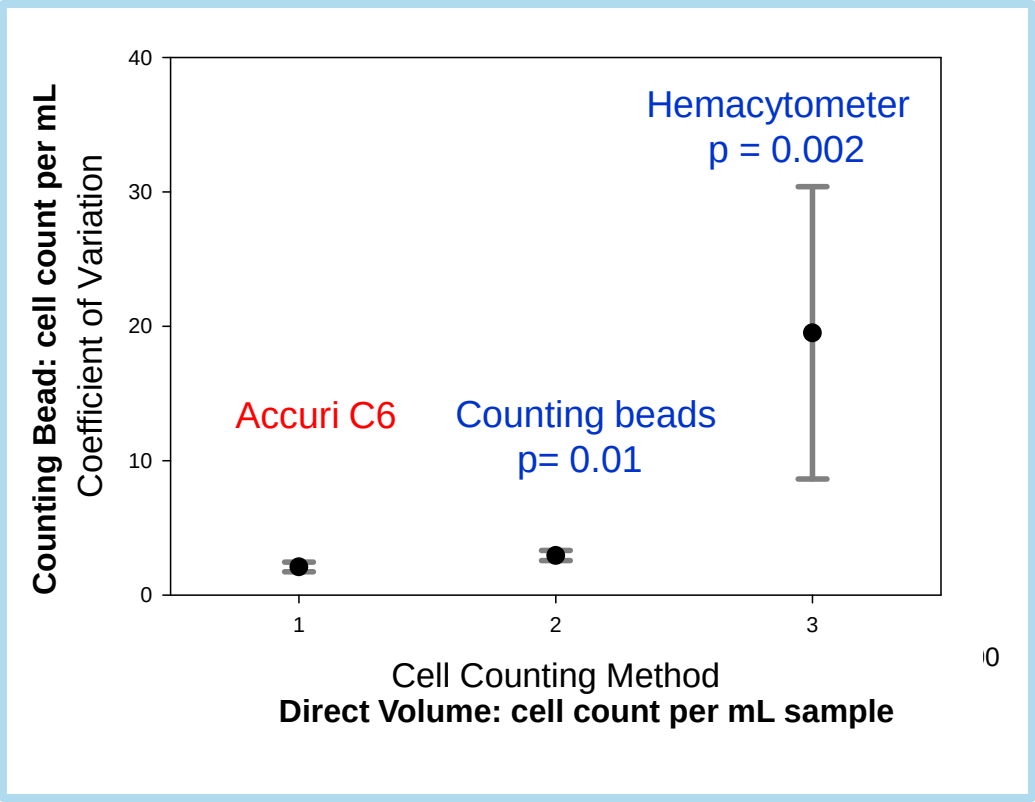
Principle of Flow Cytometry



Accuri Innovation - Fluidics

- Two peristaltic pumps operating: push-pull system
  - **Sheath Pump:** pushs fluid towards flow cell
  - **Waste pump:** pulls sheath and sample past flow cell
- Laminar flow :**hydrodynamic focusing**
- **Patented pulse dampeners**
- Volume measurement for absolute counts
- Up to 10,000 events/second

Cell Count Verification for the Accuri C6



The average coefficient of variation for replicate cell counts using three different counting methods on the same samples.

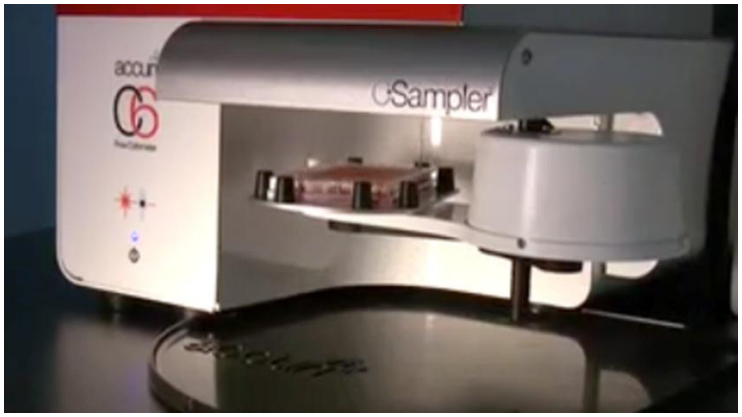
A paired student's T test was used to determine p values (95% confidence, N = 23).

C6 Maximum Event Rate = 10,000 per sec

Sample concentration	Flow rate	Approx events/sec
5 x10^6 per mL	14 µL/min	1,167
	35 µL/min	2,917
	66 µL/min	5,500

Unique Fluidics System Simplifies Sample Handling

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



Automated Flow Cytometry. Now It's Easy.

CSampler®

- Add-on automation for the C6 Flow Cytometer
- Processes 96-well and 48-well plates
- 24-position rack for 12 x 75 mm tubes
- Priority interrupt for urgent samples
- Easy-to-use software

Accuri Innovation – Pre-optimized and locked-down optics

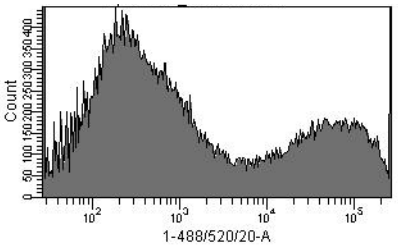
- Cylindrical flow cell
- 488 nm solid state laser
  640 nm diode laser
- Allows for fluorescence detectors at any location
  - No dichroic mirrors utilized between flow cell and detectors
  - Increased signal strength to PMT
  - Reduces alignment issues
- Results in a very stable system that is easy to transport without alignment issues

Advantages of Pre-optimzed Detector Settings

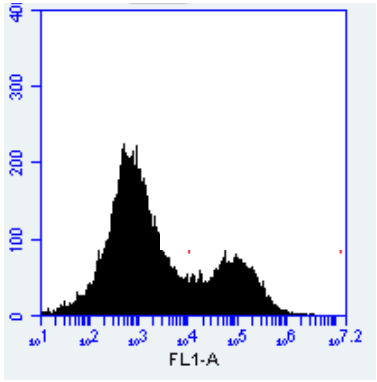
- No specialist training or dedicated operator required
- Predictable, reproducible analysis relative to sample type and application
- Saves time and sample
- Attenuation filters (for too-bright signals) give controlled signal reduction
- Predictable fluorescence spill-over
- Focus on the *science* of measuring fluorescence, not the *art* of setting voltages

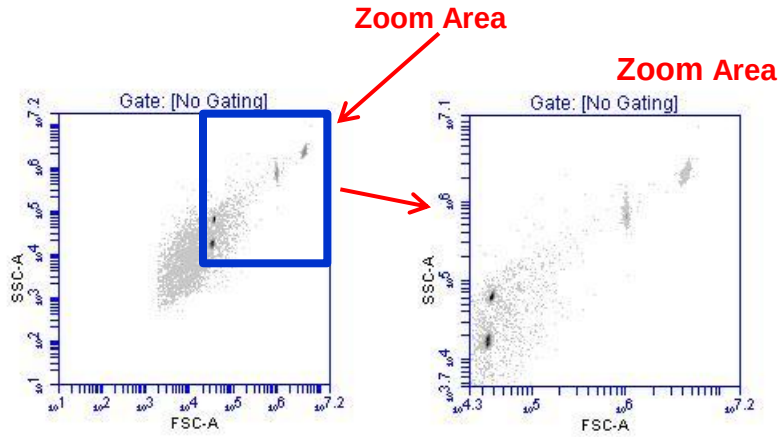
Accuri Innovation – Electronics with Broad Dynamic Range

18-Bit System



24-bit Accuri C6

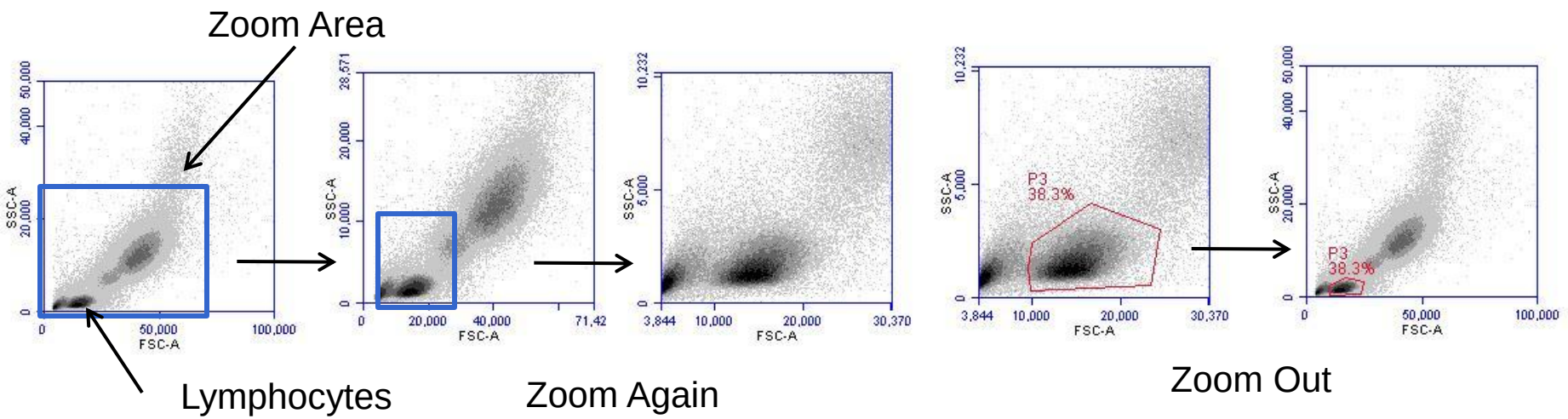




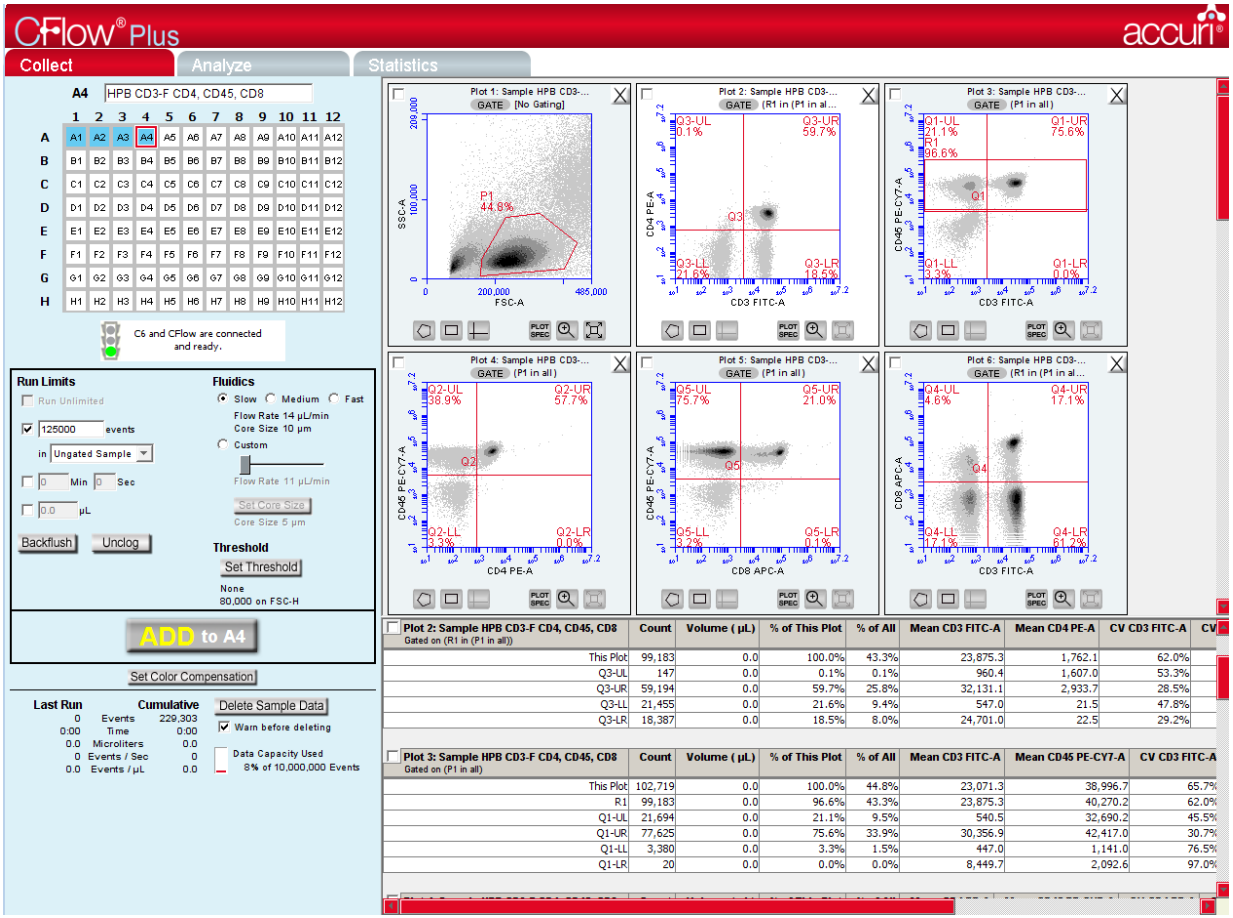
Beads: 1, 2, 12, 29 micron

More than 6 decades of signal on a single scale
All your data, available at any time

Pre-Optimized Light Scatter Detectors
Adjust Your View with Zoom



Accuri Innovation – Intuitive Software



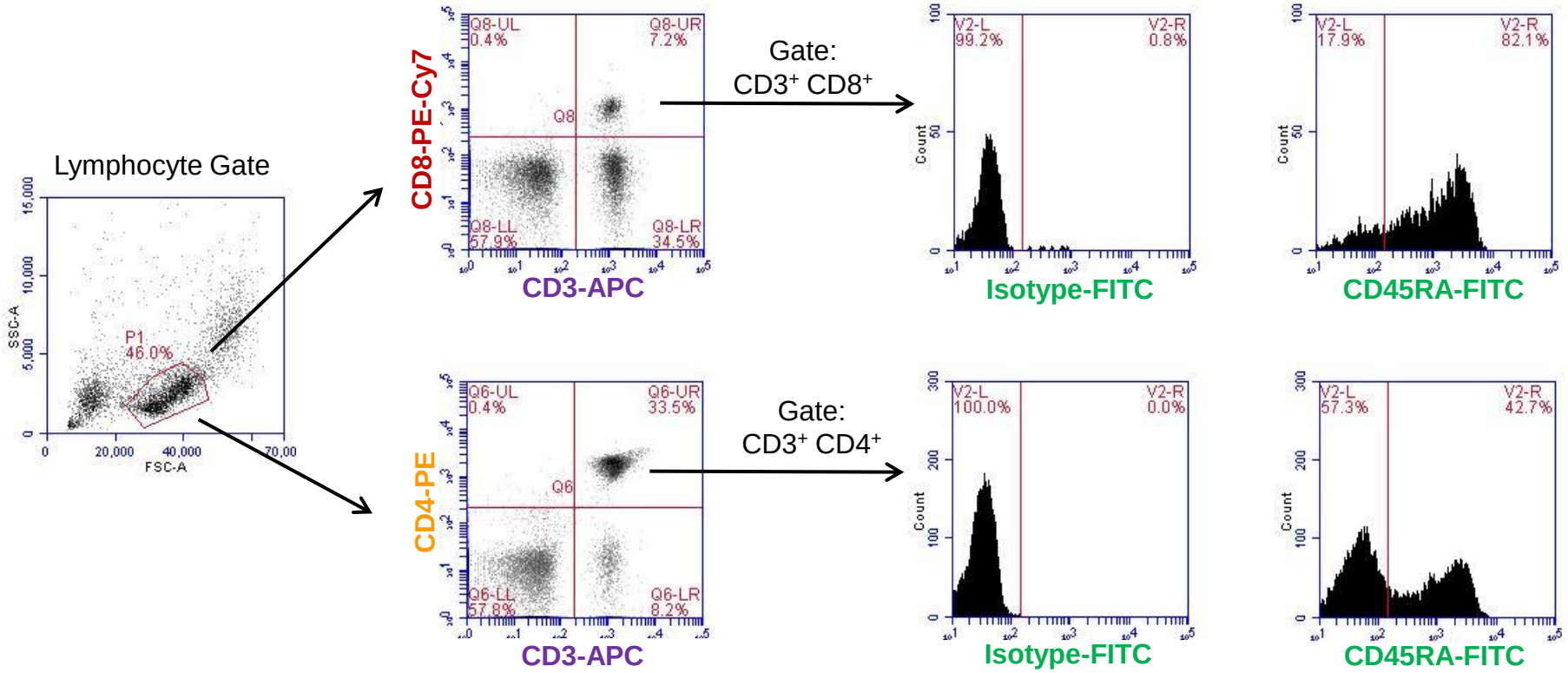
Plot 2: Sample HPB CD3-F CD4, CD45, CD8 Gated on (R1 in (P1 in all))	Count	Volume (µL)	% of This Plot	% of All	Mean CD3 FITC-A	Mean CD4 PE-A	CV CD3 FITC-A	CV
This Plot	99,183	0.0	100.0%	43.3%	23,875.3	1,762.1	62.0%	
Q3-UL	147	0.0	0.1%	0.1%	960.4	1,607.0	53.3%	
Q3-UR	59,194	0.0	59.7%	25.8%	32,131.1	2,933.7	28.5%	
Q3-LL	21,455	0.0	21.6%	9.4%	547.0	21.5	47.8%	
Q3-LR	18,387	0.0	18.5%	8.0%	24,701.0	22.5	29.2%	

Plot 3: Sample HPB CD3-F CD4, CD45, CD8 Gated on (P1 in all)	Count	Volume (µL)	% of This Plot	% of All	Mean CD3 FITC-A	Mean CD45 PE-CY7-A	CV CD3 FITC-A
This Plot	102,719	0.0	100.0%	44.8%	23,071.3	38,996.7	65.7%
R1	99,183	0.0	96.6%	43.3%	23,875.3	40,270.2	62.0%
Q1-UL	21,694	0.0	21.1%	9.5%	540.5	32,690.2	45.5%
Q1-UR	77,625	0.0	75.6%	33.9%	30,356.9	42,417.0	30.7%
Q1-LL	3,380	0.0	3.3%	1.5%	447.0	1,141.0	76.5%
Q1-LR	20	0.0	0.0%	0.0%	8,449.7	2,092.6	97.0%

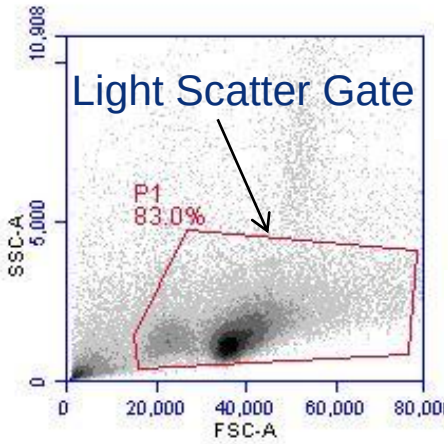
Applications

Life Science	• Immunophenotyping • Cellular processes: viability, apoptosis, membrane potential changes, ROS, ion fluxes • Cell counting • Protein expression and activation • DNA content/ploidy/cell cycling • Cytokine detection – intracellular and excreted • Multiplexed protein quantitation (BD CBA beads)
Clinical Research	• Disease diagnosis/ treatment monitoring • Cord and blood product quality
Industry	• Bacteria, yeast detection, viability, quantitation • Algae/ phytoplankton analysis

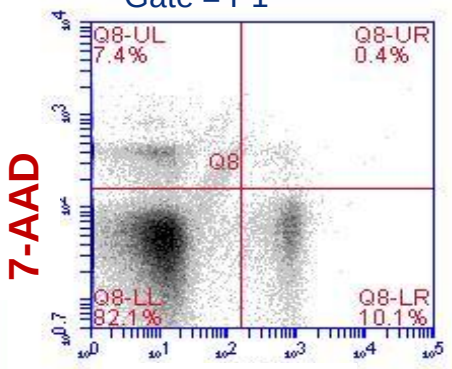
Human PBMC : T cell Phenotyping



Absolute counts of viable CD8 and CD4 T cells: Mouse splenocytes

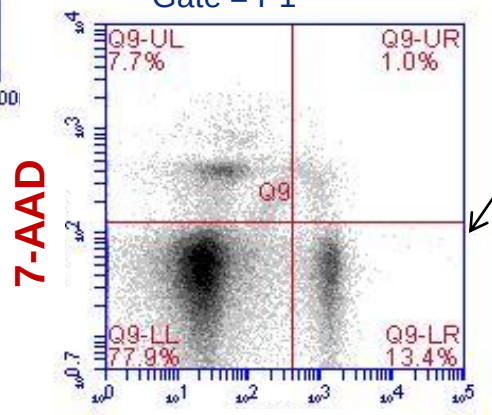


Gate = P1



CD8a PE-A

Gate = P1



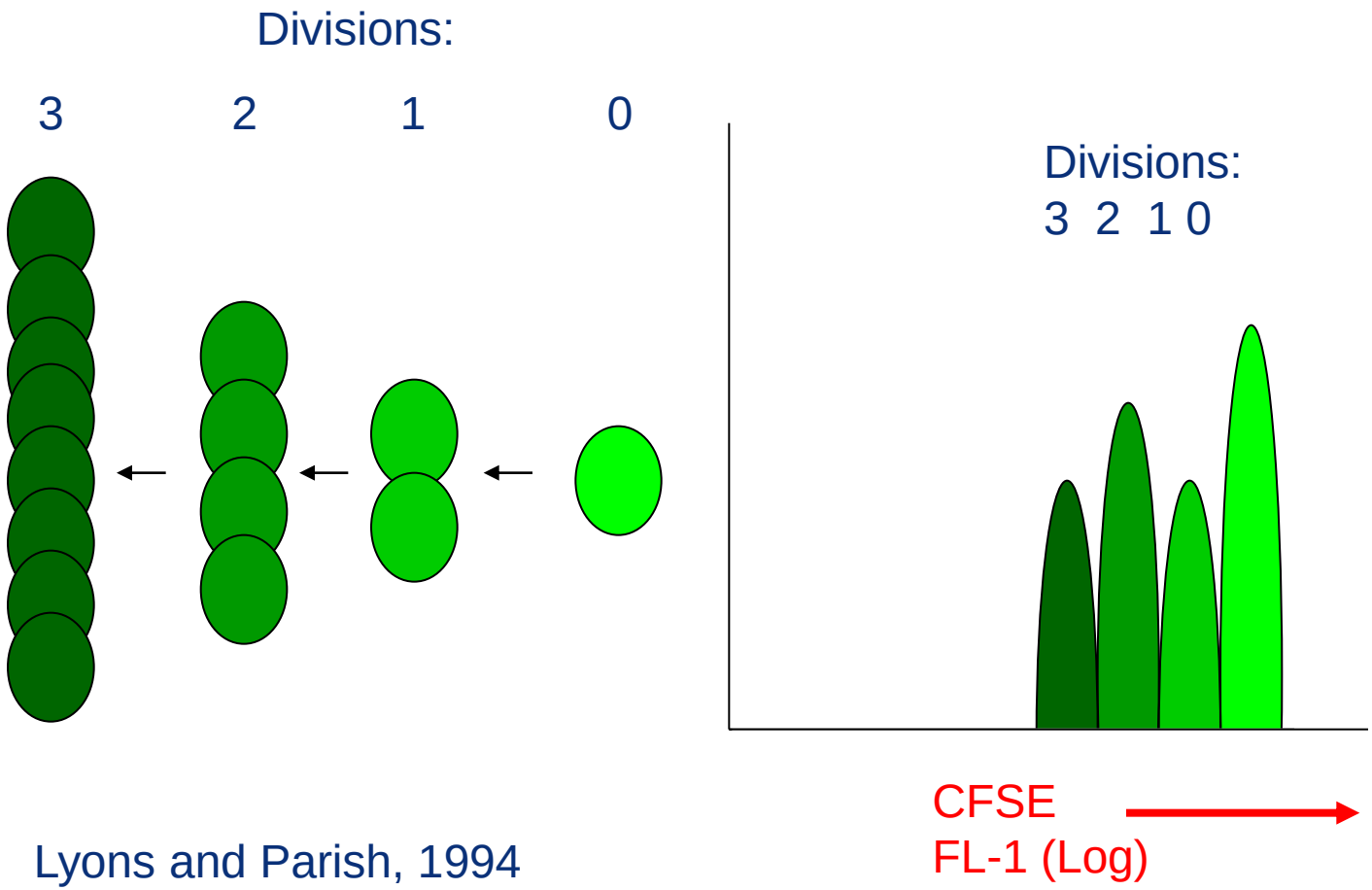
CD4 FITC-A

Viable T Cells: Lower Right Quadrant

Gated on (P1 in all)	Count	Volume (µL)	Cells/µL	Phenotype
This Plot	58,588	6.4	9154	**Total Cells in P1**
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**

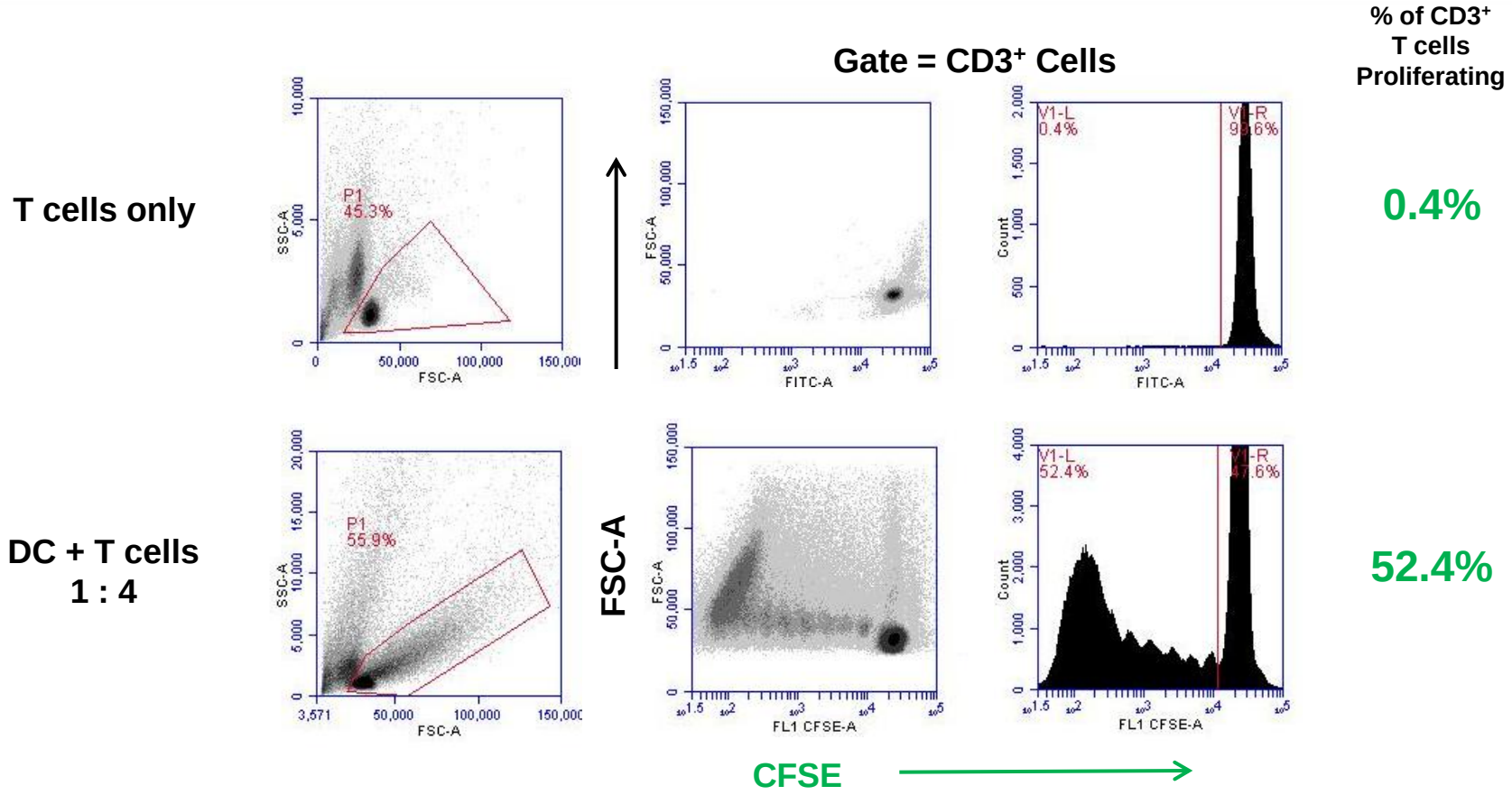
Gated on (P1 in all)	Count	Volume (µL)	Cells/µL	Phenotype
This Plot	58,588	6.4	9154	**Total Cells in P1**
Q9-UL	4,495	6.4		
Q9-UR	571	6.4		
Q9-LL	45,647	6.4		
Q9-LR	7,875	6.4	1230	**Viable CD4⁺ Cells**

Proliferation Assay--CFSE



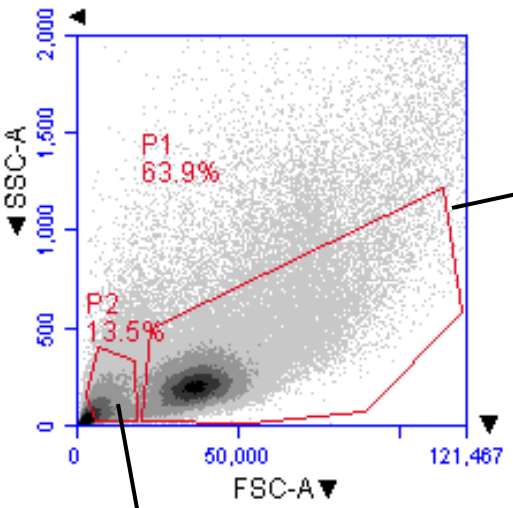
Lyons and Parish, 1994
JIM, 171;131

In Vitro T cell proliferation: T cells + dendritic cells (DC)

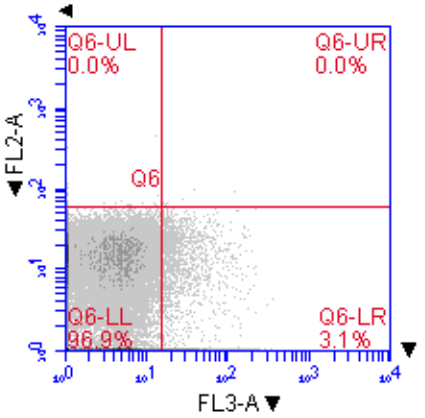


Courtesy of: Reddy P, Sung Y. Department of Pediatrics, University of Michigan, Ann Arbor, MI

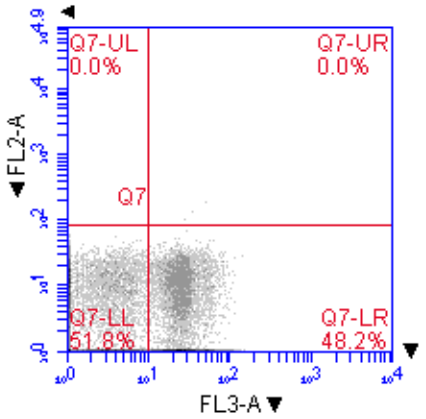
Use of 7-AAD for Viability Determination



Viable cells within higher FSC region are impermeable to 7-AAD



Dead cells within lower FSC region are permeable to 7-AAD

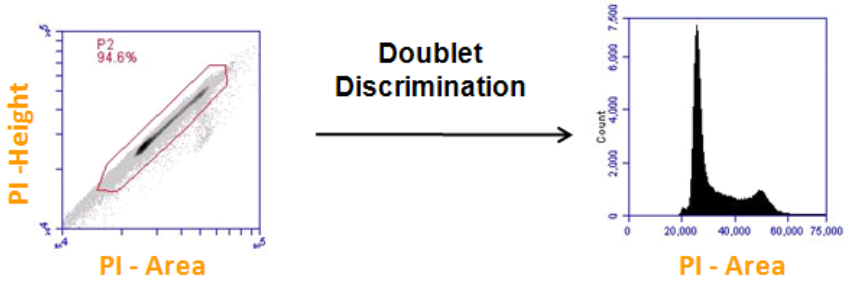


7-AAD

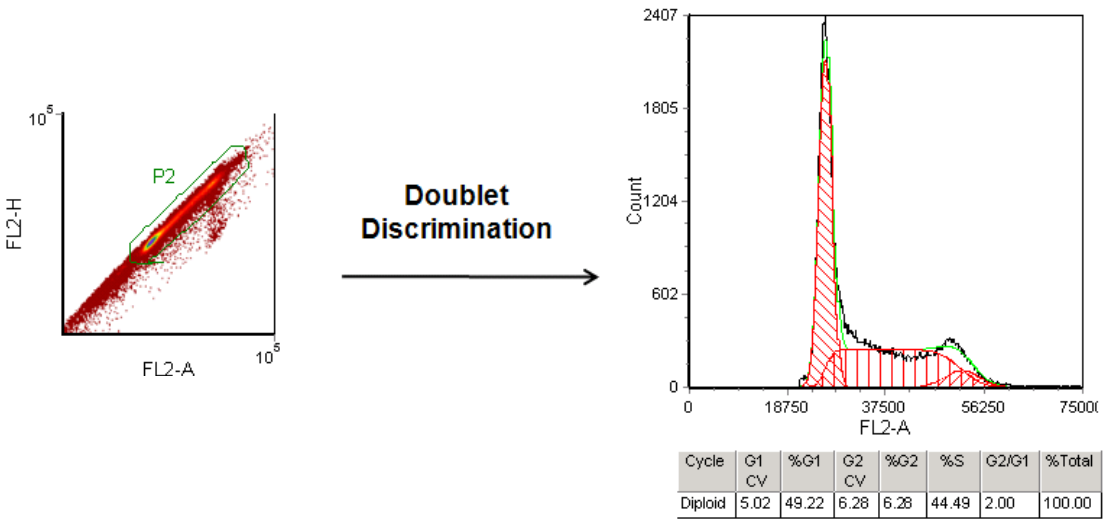
Methods: Splenocytes from 3 to 6-month old C57BL/6 mice were irradiated with UV light. Cell viability was determined by adding 1 µL 7-AAD from a stock solution of 1 mg/mL (BD Pharmingen™) per 100 µL volume of cells.

DNA Detection and Cell Cycle Analysis

Accuri C6: Data Collection



FCS Express: Cell Cycle Analysis



Cycle	G1 CV	%G1	G2 CV	%G2	%S	G2/G1	%Total
Diploid	5.02	49.22	6.28	6.28	44.49	2.00	100.00

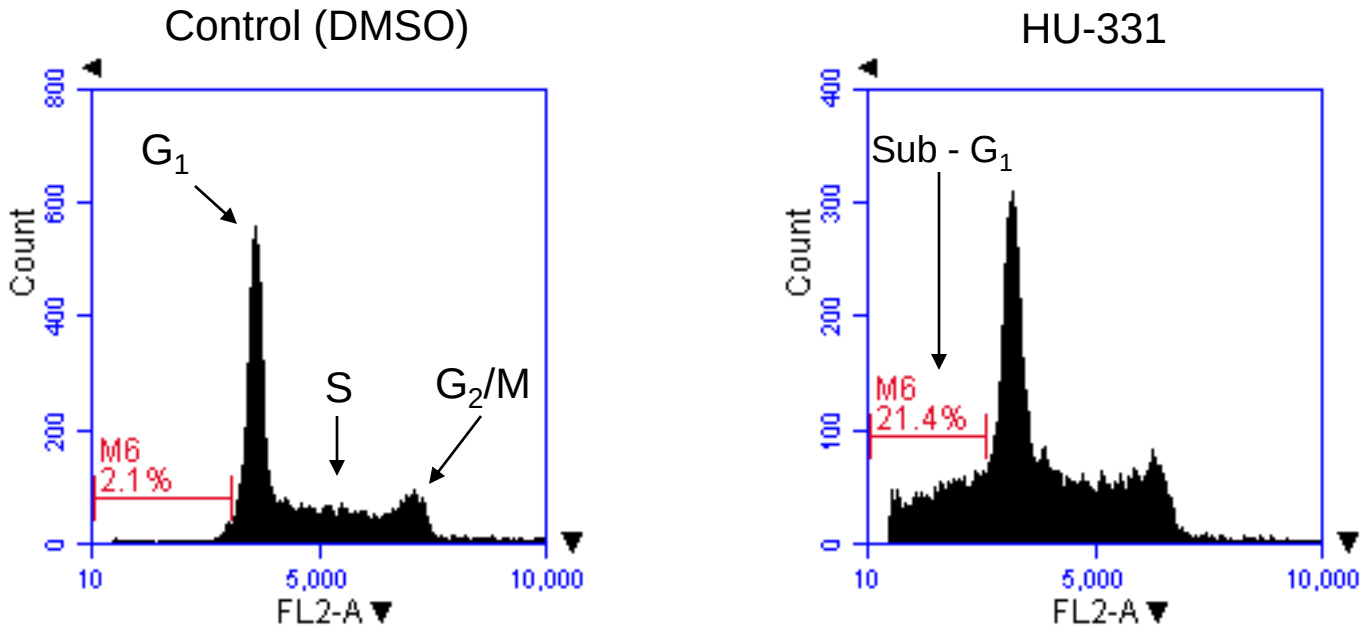
Data File Format:

- FCS 3.0 compliant

Compatible with:

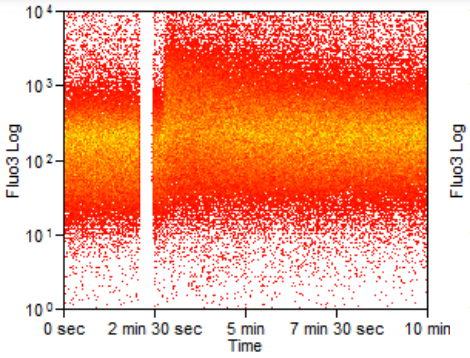
- FCS Express: CFlow File Importer
- MultiCycle
- FlowJo 7.6
- Winlist™ and Modfit LT™
- VenturiOne®

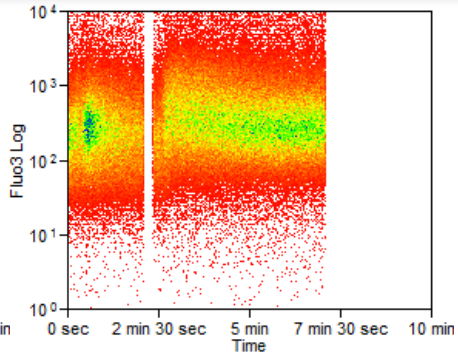
DNA Staining with Propidium Iodide

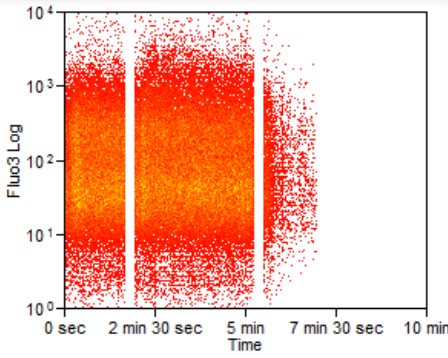


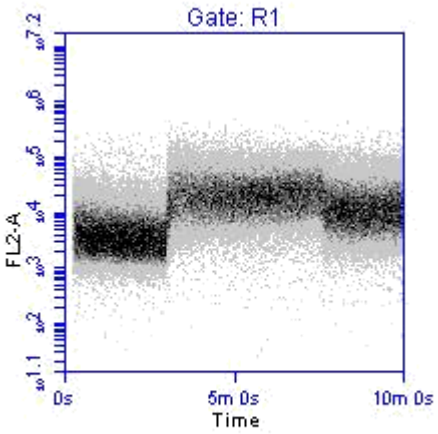
Methods: Jurkat cells were treated with either DMSO (Control; <2%) or HU-331, an apoptosis inducing cannabinoid (5 μg/mL), Cayman Chemical Company, for 6 hours. Cells were then fixed and stained with Accuri Cell Cycle Phase Determination Kit (KR-300).

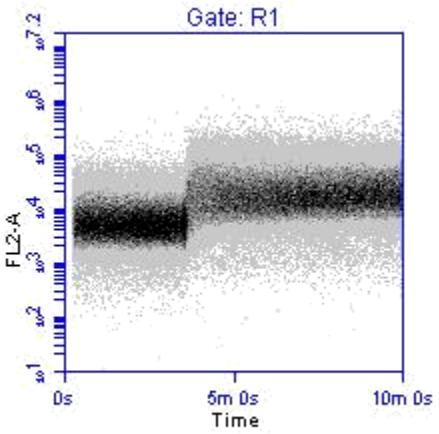
Calcium Flux Measurement with Fluo-4

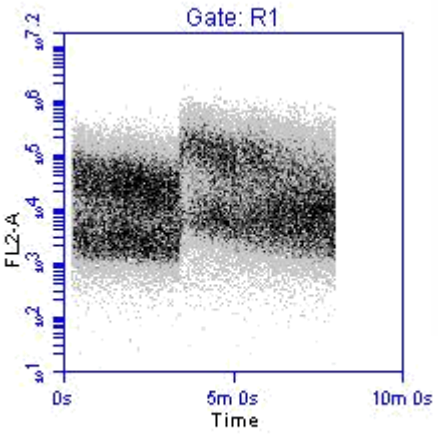








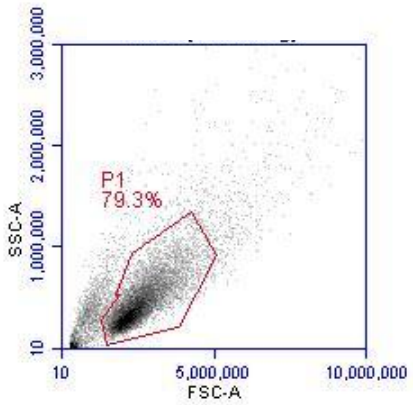


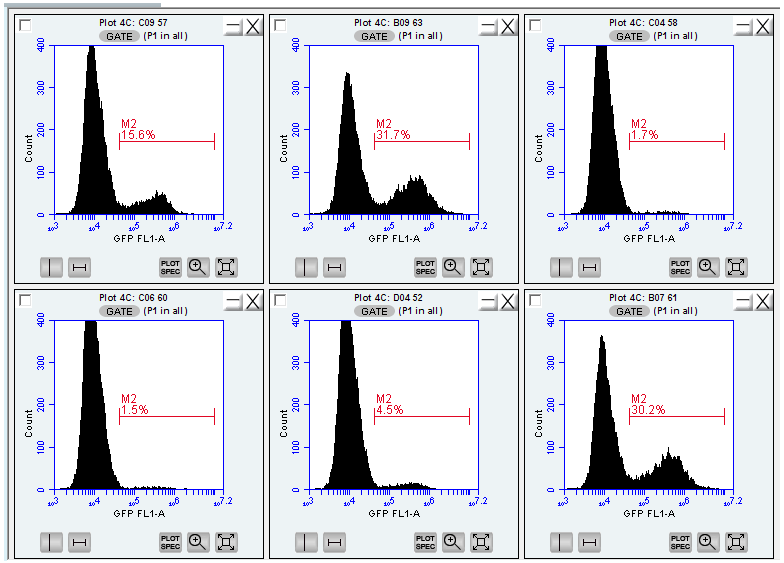


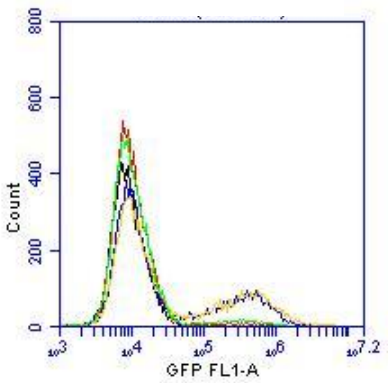
Sample tubes on the C6 do not require pressurization. Agonists can be added during sample acquisition, ensuring that one is able to visualize the entire kinetic activity.

Fluorescent Protein Detection with the Accuri C6

Transfection Screening





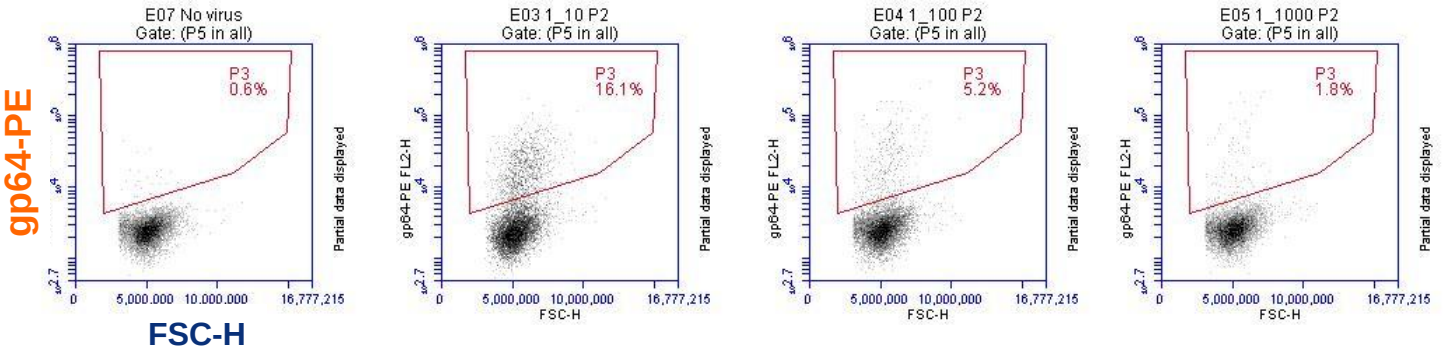


GFP ⟶

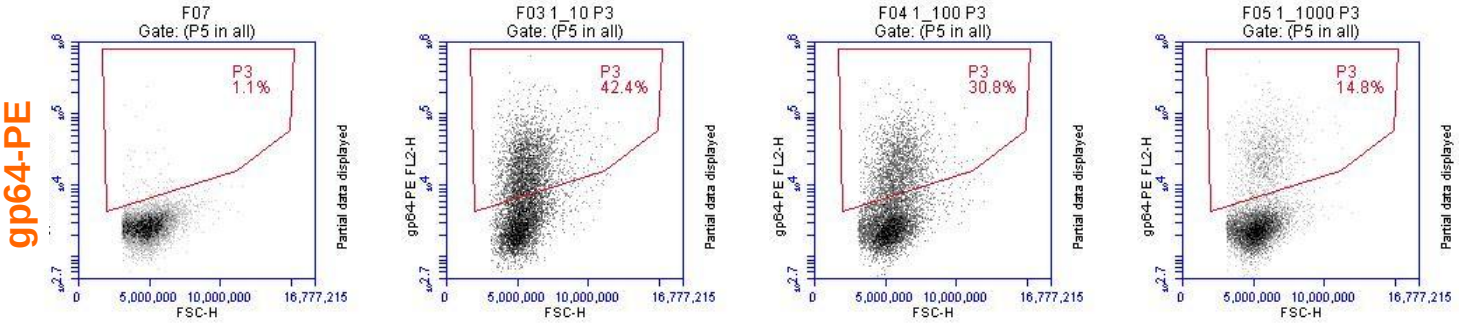
gp64-PE Expression in SF9 Insect Cells Infected with Baculovirus.

Virus Generation ↓

P2



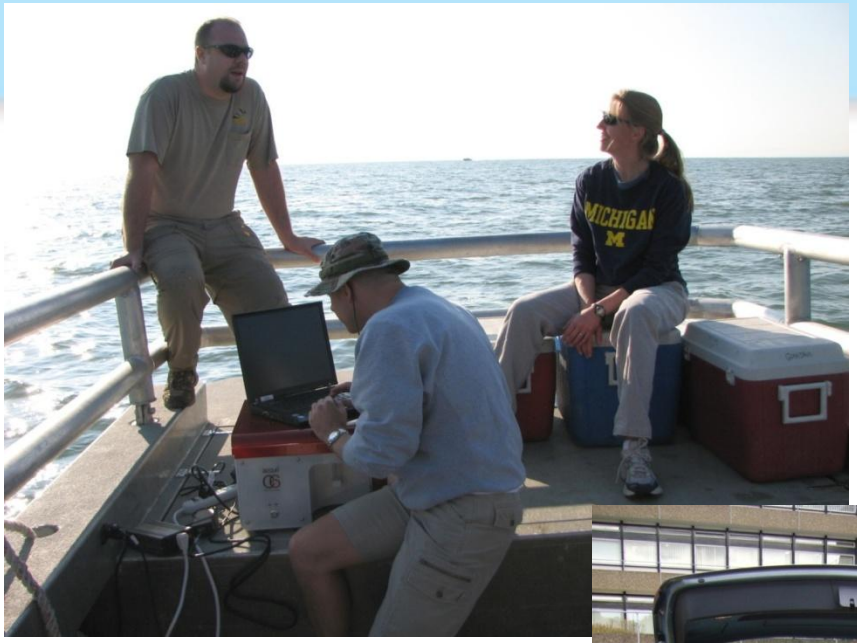
P3



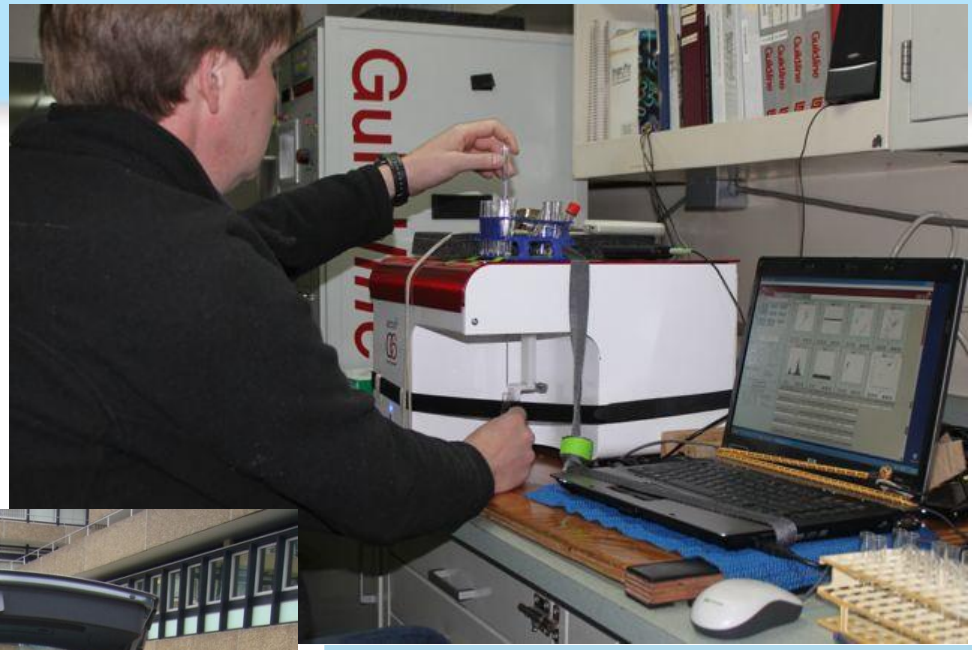
Viral Dilution →

No virus | 1:10 | 1:100 | 1:1000

The Accuri C6 is Robust!



National Oceanic and Atmospheric Administration:
Great Lakes Microcystis research project – Lake Erie



The Ecosystem Centre:
Palmer Peninsula, Antarctica

2nd Norwich Flow Day:
Back of Kate's car, Institute for Food Research

Thanks for Your Attention

許博彥 **Charlie Hsu**

Instrument Product Specialist

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