BD Accuri™ C6 Flow Cytometer

Mirko Corselli, PhD
BD Biosciences
Senior Scientist
The BD Accuri C6 Flow Cytometer System

- An affordable, full-featured, easy-to-use flow cytometer
- Two lasers and six detectors

Flow Cytometry within Reach™
The BD Accuri™ C6 Personal Flow Cytometry Tour
BD Accuri C6 Flow Cytometer

Output

Statistical Analysis

Flow Cytometry within Reach™
The BD Accuri™ C6 Personal Flow Cytometry Tour
Advantages of Pre-optimized Detector Settings

• Greatly reduces the risk of lost data due to improper setup

• Saves time and sample

• No specialist training or dedicated operator required

• Predictable, reproducible analysis relative to the sample type and application
Enhanced Sample Handling

- Direct volume measurement
- Many types of sample tubes may be used.
  - Flow cytometry tubes
  - Microcentrifuge tubes
  - Ninety-six-well plates with the BD CSampler™ accessory
- Open system conducive to kinetic studies
- BD CSampler™ accessory for automated sample introduction
Intuitive Software

Flow Cytometry within Reach™
The BD Accuri™ C6 Personal Flow Cytometry Tour

Sample Grid
Cytometer Status
Fluidics Controls
Run Criteria
Real-Time Updates

Histogram, Dot Plot, and Density Plot Display Area
Analysis and Gating Tools
Plot Statistics

Histogram, Dot Plot, and Density Plot Display Area
Analysis and Gating Tools
Plot Statistics

Flow Cytometry within Reach™
The BD Accuri™ C6 Personal Flow Cytometry Tour
A Versatile Instrument for Broad Applications

Microbiology
- Aquatic microbiome analysis
- Biofuel research
- Bacteria viability and concentration

Plant Biology
- DNA content

Cell Biology
- Apoptosis
- Proliferation
- Immunophenotyping

Fluorescent Protein Analysis
- GFP, YFP
- mCherry®, RFP
- mOrange®, dTomato®

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Jurkat cells were treated with DMSO or camptothecin for 4 hours.

**Live cells:**
- Annexin V Negative
- PI Negative

**Early apoptotic:**
- Annexin V Positive
- PI Negative

**Late apoptosis/dead:**
- Annexin V Positive
- PI Positive

Flow Cytometry within Reach™
The BD Accuri™ C6 Personal Flow Cytometry Tour
Four types of phytoplankton were identified by fluorescence characteristics.

Data courtesy of J.D. Bressie, PhD, NOAA, Seattle, WA
### Kits and Templates on the BD Accuri C6

<table>
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<th>Kit</th>
<th>Cat. No.</th>
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**Flow Cytometry within Reach™**
The BD Accuri™ C6 Personal Flow Cytometry Tour
The BD Accuri C6 Promotion and Personal Flow Cytometry Tour

Note:
US Region Only

The BD Accuri C6 Personal Flow Cytometry Tour

- Introduction to Flow Cytometry
- Cancer and Cell Biology Applications
- Microbial Analysis

Flow Cytometry within Reach™
The BD Accuri™ C6 Personal Flow Cytometry Tour
For Additional Information…

wwwbdbiosciencescomresourcesaccuri

Technical Support:
Ph: 877-232-8995, Prompt 3, 2
e-mail: ResearchApplications@bd.com

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Flow cytometry in undergraduate education

Melanie Gubbels Bupp, Assistant Professor of Biology

Whitney Edwards

Samantha Scott, Talbot Weston, Sarah Murphy, Melanie Gubbels Bupp – Autumn Immunology Conference 2014
General Advice on using cytometry in higher education

• Use the technology for projects that allow students to...
  o more firmly grasp basic concepts
  o test something of interest to them

• Only tell them what they need to know about the technology WHEN they need to know it.
  o Front-loading a lot of technical information is overwhelming and often, ineffective
Examples of using cytometry at R-MC

- **Peritonitis Lab** in Immunology course for biology majors [simplified version taught in a freshman mixed (majors/non-majors) lab]

- **Phagocytosis Lab** in Immunology course for biology majors

- **Independent research projects** for undergraduate students
**Introduce students to the lab**

**Thioglycollate-Induced Peritonitis:** Recruitment of leukocytes from the circulation, and their subsequent influx into the sites of inflammation is critical for host defense and wound healing. This is a multistep process, which is regulated, in part, by adhesion molecules and chemokines that are upregulated during inflammation. An intra-peritoneal injection of thioglycollate generates local inflammation and initiates the migration of inflammatory cells to the site of inflammation. Thus, thioglycollate-induced peritonitis in mice mimics an acute inflammatory response in the peritoneum.

**Learning Objectives**

- Be able to identify particular white blood cells (lymphocytes, monocytes, neutrophils) in a blood smear.
- Explain how and why blood differentials are taken.
- Describe the chronological order in which immune cells arrive at sites of inflammation, such as the peritoneal cavity in thioglycollate-treated mice.
Peritonitis Lab Timeline

1. Learn how to perform and read blood differentials (untreated mice)

2. Inject mice with TG (1 hr timepoint); Blood differentials on all TG samples; count & stain peritoneal cells from control and TG mice

3. Finish blood differential analysis; acquire cells on BD Accuri C6; data analysis

4. Group presentations on lab + discussion questions

**Weekly Lab Session**

Inject mice with 6% thioglycollate (TG) in PBS or control PBS to induce peritoneal inflammation
R-MC Immunology students acquiring their samples

Victoria Robinson

Lauren Philips, Jane Oh, & Casey Kaufman
Explaining how cytometry works

DN, DP, CD4+, CD8+

Labeled antibody specific to CD4
Labeled antibody specific to CD8

Each dot is a cell

Flow Cytometer

Thymus
Example student data

PBS Control

1 hour post-TG

1 day post-TG

2 days post-TG
Example student data

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- **PBS Control**: 21.2% Neutrophils, 2.0% Macrophages
- **1 hour post-TG**: 2.1% Neutrophils, 16.4% Macrophages
- **1 day post-TG**: 38.9% Neutrophils, 2.1% Macrophages
- **2 day post-TG**: 20.6% Neutrophils, 1.1% Macrophages
Example discussion questions for student peritonitis presentations

• What types of junctions exist between endothelial cells lining blood vessel walls in the non-inflamed, resting condition? How do these junctions change during local inflammation?
• Relate your findings from the blood differentials with your findings from the flow cytometry data. Do the two sets of data “paint the same picture”? Why or why not?
• Map the route newly developed neutrophils must take to enter the inflamed peritoneal cavity. Begin in the bone marrow and end with the peritoneal cavity.
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**Learning Objectives**

- Compare and contrast monocytes and macrophages
- Evaluate flow cytometry data regarding the ability of cells to phagocytose fluorescently tagged antigens
- Be able to design an experiment to test the impact of various compounds on phagocytosis
Phagocytosis Lab Timeline

1. Learn how to culture THP-1 cells; design experiment to test effect of particular substance on phagocytosis (in vitro)
2. Incubate THP-1 cells with FITC-latex beads, and acquire samples on BD Accuri C6; data analysis
3. Group presentations on lab + discussion questions

Weekly Lab Session

Set up THP-1 cultures in 24 well plates +/- PMA and +/- test substance
Explaining how cytometry works -- histograms

Lots of phagocytosis

Very little phagocytosis

Flow Cytometer

FITC - beads

Count

FITC-beads

Count

M1 76.1%
Example student data

THP cells

0%
0 PMA

FITC-beads

THP cells + FITC-beads

45.3%

THP cells + FITC-beads + Vitamin C

52.3%

45.3%

0 PMA

FITC-beads

THP cells

M1 0.0%

0%

0% 76.1%

THP cells + PMA

M1 78.1%

76.1%

THP cells + PMA

M1 66.5%

66.5%
Examples of using cytometry at R-MC

• **Peritonitis Lab** in Immunology course for biology majors [simplified version taught in a freshman mixed (majors/non-majors) lab]

• **Phagocytosis Lab** in Immunology course for biology majors

• **Independent research projects** for undergraduate students
Students at R-MC also use the cytometer in independent research projects.
Students at R-MC also use the cytometer in independent research projects

Sarah Murphy

Isolate Naïve CD8+ T cells from Donor mice

Adoptively Transfer cells into recipients

Sort them on CD127 expression

Assess total numbers and CD127 expression of CFSE+ cells by flow cytometry

MAL

AL

2.5 x 10^6

2.5 x 10^6

CFSE^{hi}

CD127^{lo}

CFSE^{lo}

CD127^{hi}

1 week
Students at R-MC also use the cytometer in independent research projects.

Naïve CD8+ CFSE+ T Cells in recipient LNs

Donor cells: CD127^{low} CD127^{high}
Thank you to R-MC students participating in labs and independent research

- Whitney Edwards
- Erica Horseman
- Rebecca Davis
- Seth Litvin
- Victoria Robinson
- Josh Anoff
- Brittany Mihalcoe
- Alex Koppleman
- Samantha Scott
- Sarah Murphy
- Talbot Weston

Talbot Weston, Sarah Murphy, and Samantha Scott
Integrating *Microbial* Flow Cytometry Into Education

Tim W Overton
Bioengineering, School of Chemical Engineering
University of Birmingham

t.w.overton@bham.ac.uk  @overtonlab
How can we get FCM applied in microbiology / microbial biotechnology?

- Collaboration between FCM specialists and microbiology / bioprocessing researchers

- Training at University level
Teaching fermentation

• MSc Biochemical Engineering
  – Fermentation and cell culture for production of biopharmaceuticals
  – Downstream processing of biopharmaceuticals
  – Systems and synthetic biology
  – Pharmaceutical, food and business themes
  – Research project
Teaching analysis of fermentation

• Theory sessions on analytical techniques
  – Online versus offline
  – Real time / non-real time
  – Bulk versus single cell
  – Direct observations
• Theory and advantages of FCM
Lab-scale fermentation

- Growth of *E. coli* in 5 litre bioreactors
- Analysis:
  - Traditional techniques: pH, DOT, offgas
  - Biomass measurements:
    - Optical density
    - Colony forming units
    - Dry cell weight
  - FCM
Fermentation data

Traditional techniques:
Dissolved O$_2$ and pH
Basics of growth and physiology

O$_2$ consumption,
CO$_2$ evolution
Basics of growth and physiology
FCM with physiology dyes

1 hour

Propidium Iodide (PI)
Enters cells through holes in wall
Stains dead cells red

DiBac$_4$(3) (Bisoxanol; BOX)
Only enters depolarised cells
Stains depolarised cells green

Advantages:
• Rapid method
• Allows monitoring of VBNC bacteria
• Allows counting of cells
FCM with physiology dyes

1 hour

2 hours

3 hours

4 hours

5 hours

6 hours
Pilot plant

- Growth of *E. coli* in 150 litre vessels over the course of a week
- Fed-batch with glucose
  - Glucose feeding rate is critical to success
- Students encouraged to develop their own analysis methods and strategies – including FCM
Team 1 – FCM physiology monitoring

More dead and injured cells – reflects poor glucose feed control

Team 2 – FCM physiology monitoring

More healthy cells – good glucose feed control
Food microbiology

- Use of flow cytometry as a method for monitoring bacteria during food processing
- Acid resistance and acid adaptation
- Differentiation between bacteria and food matrix
MSc / MEng Research projects

- Monitoring physiology using dyes during biotransformation reactions
- Monitoring poly(3-hydroxybutyrate) (PHB) productivity by bacterial cultures
- Recombinant protein-GFP fusion studies to screen new growth conditions such as choice of carbon source
  - RP-GFP fusions allow measurement of both quantity and folding quality
Doctoral training

• BBSRC-funded MIBTP (Midlands Integrative Biosciences Training Partnership)
  – Warwick, Birmingham & Leicester Universities
  – 52 PhD students per year

• Extensive training programme:
  – Quantitative skills
  – Placements
  – *Techniques masterclasses*
Doctoral training - master class

• Day 1:
  – Day of lecture / seminar teaching
  – Ability to go into detail and give examples
  – Papers given to students

• Day 2: Reading / preparation

• Day 3:
  – Students give 10 minute talks on papers
  – Practical session
Doctoral training - practical

- *E. coli* wild-type
- *E. coli* GFP+
- *S. cerevisiae*
- Comparison of scatter measurements
- Live & Dead cells with a variety of stains (PI, BOX, SYTO9)
- Unknown mixtures (problem-solving aspect)
- Hands-on time is invaluable to apply theory (and test theory)
Challenges and opportunities

• Students have diverse backgrounds
  – Gaps in knowledge (e.g. optics, fluorescence)
  – Students can teach each other

• Students are interested in different areas
  – Comparisons with other techniques
  – Fertile ground for new ideas about methods and measurement techniques

• Students are very keen!
Outlook

• Training in theory of FCM is interdisciplinary and needs life science and physical science knowledge.

• Comparison with previously-used techniques is helpful.

• Combination of taught material with practical experience is very useful.
Acknowledgements

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