How to Analyze More Markers Without Adding Detectors

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Outline

• Taking Advantage of Biology
  – Examples of using SSC to separate populations

• Taking Advantage of Spillover
  – Explanation of techniques
  – Taking advantage of fixed voltage
  – Examples of expanding 3 markers on 2 detectors to 7 markers on 4 detectors

• Summary – Biological Situations You Can Try to Exploit

• Available Resources
Taking Advantage of Biology

- **BD Phosflow™ Human Monocyte/NK Cell Activation Kit**
  - FL1 = CD14 Alexa Fluor® 488 – Monocytes
  - FL1 = CD19 Alexa Fluor® 488 – B Cells
  - FL2 = CD3 PE-Cy™7 – T Cells
  - FL3 = CD16 PE – NK Cells
  - FL3 = CD56 PE – NK Cells
  - FL4 = Stat3 (pY705) Alexa Fluor® 647

(Cat. No. 562089)
Taking Advantage of Biology

BD Phosflow Human Monocyte/NK Cell Activation Kit
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Taking Advantage of Biology

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Taking Advantage of Biology

BD Phosflow Human Monocyte/NK Cell Activation Kit
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Taking Advantage of Spillover

Population A

Population B

Population C
BD Accuri™ C6 Flow Cytometer System

- An affordable, fully-featured, easy-to-use flow cytometer
- Two lasers and six detectors
Preset Voltages on the BD Accuri C6
Increase Data Reproducibility
Preset Voltages on the BD Accuri C6 Lead to Predictable Spillover
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<table>
<thead>
<tr>
<th>Fluorochrome</th>
<th>Equation</th>
<th>Correlation</th>
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</thead>
<tbody>
<tr>
<td>FITC into FL2</td>
<td>$y = 0.0734x + 311$</td>
<td>$r^2 = 0.98$</td>
</tr>
<tr>
<td>PE into FL1</td>
<td>$y = 0.037x + 983$</td>
<td>$r^2 = 0.97$</td>
</tr>
<tr>
<td>APC into FL3</td>
<td>$y = 0.007x + 643$</td>
<td>$r^2 = 0.96$</td>
</tr>
<tr>
<td>PE-Cy™5 into FL4</td>
<td>$y = 0.4613x + 6016$</td>
<td>$r^2 = 0.98$</td>
</tr>
<tr>
<td>PE-Cy7 into FL4</td>
<td>$y = 0.0013x + 480$</td>
<td>$r^2 = 0.19$</td>
</tr>
</tbody>
</table>
Taking Advantage of Spillover
7 markers on 4 detectors

- CD16 + CD56 PE-Cy7 – NK Cells
- CD19 APC – B Cells
- CD3 PE-Cy5 – T Cells
- CD4 APC – Helper T Cells
- CD8 PE – Cytotoxic T Cells
- IgD PE – Mature B Cells
- CD127 FITC – Regulatory T Cells
Taking Advantage of Spillover
3 markers on 2 detectors

B cells: CD19 APC

NK cells: CD56+16 PE-Cy7

T cells: CD3 PE-Cy5

B01 7
Gate: Lymphs

A05 5
Gate: Lymphs

A06 6
Gate: Lymphs

E All 3 cell types

CD19 APC

CD3 PE-Cy5

CD56 PE-Cy7

CD16+56 PE-Cy7

BD
Adding a Double Positive Population

4 markers on 2 detectors

CD19 APC, CD16 + CD56 PE-Cy7, CD3 PE-Cy5, CD4 APC
Adding Subsets of Different Populations

7 markers on 4 detectors

CD19 APC, CD16 + CD56 PE-Cy7, CD3 PE-Cy5, CD4 APC, CD8 PE, IgD PE, CD127 FITC
Controls Confirm That CD8 and IgD Stain Different Populations
Summary – Biological Situations You Can Try to Exploit

- Mutually exclusive populations
- Subsets of different populations
- Differences in intensity of signals
- Differences in scatter properties—i.e., size or granularity of cells

Always run controls to verify the biology and gating.
Resources: Technical Bulletin and Multicolor Flow Cytometry Tools

- **Taking Advantage of Fluorescence Spillover to Analyze More Markers on Fewer Detectors: Thinking Outside the Orthogonal Box** (Technical Bulletin)

  bdbiosciences.com/support/resources/accuri

- Multicolor Flow Cytometry Tools

  bdbiosciences.com/research/multicolor/tools
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