Design of Multicolor Flow Cytometry Panels Incorporating BD Horizon™ Brilliant Violet™ Dyes

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BD Biosciences
Outline

• BD Horizon™ Brilliant Violet™ fluorochrome characteristics:
  • Excitation/emission
  • Brightness
  • Spillover
  • Buffer compatibility

• New BD Horizon™ Brilliant UltraViolet™ fluorochrome

• Panel design considerations and examples
Brilliant Violet™ fluorochromes

BV421  BV510  BV605  BV650  BV711  BV786

Wavelength (nm)
Sirigen polymers overview

Brightness of QDot® and fluorescent proteins... with the well-defined and tunable features of dye chemistry

Direct reporters

- Many units = efficient light harvesting
- Properties controlled by size and composition
- Replaces poor performing reporters

Bright QDot® brightness... with the well-defined and tunable features of dye chemistry.
BD Horizon™ BV421: The new standard for brightness

- PE: Has been the brightest fluor available
- BV421: The new standard for brightness

<table>
<thead>
<tr>
<th>Spec.</th>
<th>µg/test</th>
<th>BV421</th>
<th>V450</th>
<th>PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>0.125</td>
<td>759</td>
<td>71</td>
<td>544</td>
</tr>
<tr>
<td>CD4</td>
<td>0.125</td>
<td>520</td>
<td>51</td>
<td>248</td>
</tr>
<tr>
<td>CD8</td>
<td>0.125</td>
<td>924</td>
<td>34</td>
<td>445</td>
</tr>
<tr>
<td>CD19</td>
<td>0.125</td>
<td>400</td>
<td>33</td>
<td>119</td>
</tr>
<tr>
<td>CD25</td>
<td>0.125</td>
<td>52</td>
<td>27</td>
<td>7</td>
</tr>
<tr>
<td>CD38</td>
<td>0.125</td>
<td>30</td>
<td>4</td>
<td>8</td>
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<tr>
<td>CD56</td>
<td>0.125</td>
<td>76</td>
<td>8</td>
<td>22</td>
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<tr>
<td>CD73</td>
<td>0.125</td>
<td>50</td>
<td>8</td>
<td>19</td>
</tr>
<tr>
<td>CD117</td>
<td>0.25</td>
<td>30</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>CD184</td>
<td>0.25</td>
<td>20</td>
<td>3</td>
<td>7</td>
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</table>

Average 11.3 3.6
• Base polymer
• Excitation maximum at 405 nm (also has UV excitation)
• Emission maximum at 510 nm
• Use in same filter as BD Horizon™ V500/V500-C/AmCyan: 525/50 nm
BD Horizon™ BV605 spectra

- Polymer-based tandem
  - BV421 + Acceptor Em 605
- Excitation maximum: 407 nm
- Emission maximum: 605 nm
BD Horizon™ BV650 spectra

- Polymer-based tandem
  - BV421 + Acceptor Em 650
- Excitation maximum: 407 nm
- Emission maximum: 650 nm
- Recommended filter: 660/20
BD Horizon™ BV711 spectra

- Polymer-based tandem
  - BV421 + Acceptor Em 711
- Excitation maximum: 407 nm
- Emission maximum: 711 nm
- Recommended filter: 710/50
**BD Horizon™ BV786 spectra**

- Polymer-based tandem
  - BV421 + Acceptor Em 785
- Excitation maximum: 407 nm
- Emission maximum: 786 nm
PERFORMANCE COMPARISON TO OTHER EXISTING DYES
BV510 is brighter than V500

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Fluor</th>
<th>Stain index</th>
</tr>
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<tbody>
<tr>
<td>Ms CD11b</td>
<td>BV510</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>V500</td>
<td>10</td>
</tr>
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<td></td>
<td>FITC</td>
<td>25</td>
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<td>Hu CD19</td>
<td>BV510</td>
<td>49</td>
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<tr>
<td></td>
<td>V500</td>
<td>17</td>
</tr>
</tbody>
</table>

Ms CD11b staining
BV510 is brighter than Qdot® 525

Hu CD4 Biotin + SAV

% of Max

0 20 40 60 80 100

0 10^2 10^3 10^4 10^5

BV510-A

BV510
Qdot® 525
BV605 is brighter and has less spillover than Qdot® 605

Excitation profile: Qdot® 605 Brilliant Violet™ 605

<table>
<thead>
<tr>
<th>Specificity</th>
<th>Clone</th>
<th>Fluorochrome</th>
<th>Stain Index</th>
<th>BD Horizon™ 450 (450/50)</th>
<th>BD Horizon™ V500 (525/20)</th>
<th>BD Horizon™ PE-CF594 (610/20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD27</td>
<td>L128</td>
<td>Brilliant Violet™ 605</td>
<td>174</td>
<td>2.1%</td>
<td>0.3%</td>
<td>5.7%</td>
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<tr>
<td></td>
<td>CLB-27/1</td>
<td>Qdot® 605</td>
<td>62</td>
<td>0.0%</td>
<td>0.0%</td>
<td>71.7%</td>
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</table>
BV711 is brighter than Qdot® 705

<table>
<thead>
<tr>
<th></th>
<th>Stain Index</th>
</tr>
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<tbody>
<tr>
<td>CD4 BV711</td>
<td>744</td>
</tr>
<tr>
<td>CD4 Qdot 705</td>
<td>93</td>
</tr>
<tr>
<td>CD4 Bio-SA BV711</td>
<td>316</td>
</tr>
<tr>
<td>CD4 Bio-SA Qdot 705</td>
<td>24</td>
</tr>
</tbody>
</table>

Hu CD4 Biotin + SAV

SAV Qdot® 705, BV711
NEW FLUOROCHROME BRIGHTNESS RANKING
BV reagents are bright

Example: CD4 staining, human LWB

- BV421
- BV711
- BV650
- BV786
- BV605
- BV510
Brightness ranking: Stain index

- Stain index absolute values will depend on:
  - Reagent, clone selection
  - Instrument configuration (laser wavelength, laser power, filters)
  - Instrument setup

- Ideally, ranking should be established for each specific instrument
Brightness ranking

**BRIGHT**
BV421, BV650, BV711, BV786
PE, PE-CF594, PE-Cy™7
APC

**MODERATE**
BV605, BV510
FITC, Alexa Fluor® 488, PerCP-Cy™5.5
Alexa Fluor® 647, Alexa Fluor® 700

**DIM**
V450, V500, AmCyan
APC-Cy7/APC-H7
INSTRUMENT CONSIDERATIONS
Violet laser configuration

- **BV711**: 690 LP, 710/50 BP
- **BV605**: 595 LP, 610/20 BP
- **BV421/V450**: 450/40 BP
- **BV510/V500**: 505 LP, 525/50 BP
- **BV650**: 630 LP, 660/20 BP
- **BV786**: 750 LP, 780/60 BP
Example of filter selection: BV711

- Main spillover is from BV650

### MFI measurements

<table>
<thead>
<tr>
<th>Fluor/filter</th>
<th>Into BV711</th>
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<tr>
<td>BV650 695/40</td>
<td>6,850</td>
</tr>
<tr>
<td>BV650 710/50</td>
<td>9,933</td>
</tr>
<tr>
<td>BV650 712/21</td>
<td>4,096</td>
</tr>
<tr>
<td>BV711 695/40</td>
<td>7,786</td>
</tr>
<tr>
<td>BV711 710/50</td>
<td>14,438</td>
</tr>
<tr>
<td>BV711 712/21</td>
<td>7,286</td>
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</tbody>
</table>

### Stain index CD4 SK3 clone

<table>
<thead>
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<th>Reagent</th>
<th>Filter</th>
<th>Stain index</th>
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<tbody>
<tr>
<td>CD4 BV711</td>
<td>695/40</td>
<td>228.47</td>
</tr>
<tr>
<td></td>
<td>710/50</td>
<td>313.17</td>
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<tr>
<td></td>
<td>712/21</td>
<td>242.53</td>
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</table>

### SOV

<table>
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<tr>
<th>Filter</th>
<th>SOV 650/711</th>
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</thead>
<tbody>
<tr>
<td>695/40</td>
<td>0.880</td>
</tr>
<tr>
<td>710/50</td>
<td>0.688</td>
</tr>
<tr>
<td>712/21</td>
<td>0.562</td>
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</tbody>
</table>
Laser power and BV reagents

![Graph showing laser power and stain index for different BV reagents.](image)
SPILOVER
Spillover between BV reagents (1)
Spillover between BV reagents (2)

<table>
<thead>
<tr>
<th>Fluor/detector</th>
<th>BV421</th>
<th>BV510</th>
<th>BV605</th>
<th>BV650</th>
<th>BV711</th>
<th>BV786</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV421</td>
<td>100.00</td>
<td>23.69</td>
<td>1.78</td>
<td>0.28</td>
<td>0.26</td>
<td>0.28</td>
</tr>
<tr>
<td>BV510</td>
<td>3.52</td>
<td>100.00</td>
<td>29.99</td>
<td>5.60</td>
<td>3.45</td>
<td>1.50</td>
</tr>
<tr>
<td>BV605</td>
<td>3.02</td>
<td>1.96</td>
<td>100.00</td>
<td>24.66</td>
<td>13.31</td>
<td>4.97</td>
</tr>
<tr>
<td>BV650</td>
<td>4.11</td>
<td>2.06</td>
<td>25.41</td>
<td>100.00</td>
<td>57.93</td>
<td>16.29</td>
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<tr>
<td>BV711</td>
<td>4.08</td>
<td>1.93</td>
<td>0.80</td>
<td>1.10</td>
<td>100.00</td>
<td>47.95</td>
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<tr>
<td>BV786</td>
<td>3.72</td>
<td>1.98</td>
<td>1.05</td>
<td>0.31</td>
<td>2.31</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Spillover calculated as ratio of MFI of secondary fluor in detector x to MFI of primary fluor in its own detector
Spillover to other detectors

• BV605 into PE, PE-CF594 (if using the yellow-green laser)

• BV650 and BV711 into PerCP-Cy5.5, Alexa Fluor® 700

• BV786 into APC-H7
BUFFER COMPATIBILITY
Buffer compatibility (1)

Brilliant Violet reagents are compatible with:

- EDTA and heparin blood collection tubes
- BD Pharm Lyse™ lysing buffer and BD FACS™ lysing solution
- PFA-based fixatives
- BD Cytofix™ fixation buffer/BD Perm/Wash™ buffer
- BD Pharmingen™ Transcription Factor Buffer Set
- BD Phosflow™ Perm Buffer III
Buffer compatibility (2): Intracellular staining

Unstimulated

CMV pp65

SEB

IL-2 BV421

IFN-γ PE

CD4

IL-2 BV421

IFN-γ PE

CD8

IL-2 BV421
BV421 does not alter cell functionality: CD107a staining

Unstimulated

CMV pp65

SEB

IFN-γ PE

IL-2 APC
NEW UV LASER FLUOROCHROME
• Excitation maximum of 347 nm, optimum for a 355-nm ultraviolet laser.
• No significant excitation by a 405, 488, 532, 561, or 640-nm laser.
• Emission maximum of 395 nm.
BUV395 brightness

BV421

CD4

SI = 690

PE

SI = 179

SI = 125

CD19

SI = 440

SI = 57

SI = 63

BD
Helping all people live healthy lives
**BUV395 spillover**

<table>
<thead>
<tr>
<th>Laser</th>
<th>BUV395 SOV into other channels</th>
</tr>
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<tbody>
<tr>
<td>Violet</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BV421</td>
</tr>
<tr>
<td>BUV395</td>
<td>0.2%</td>
</tr>
<tr>
<td>Blue</td>
<td>FITC</td>
</tr>
<tr>
<td>BUV395</td>
<td>0.0%</td>
</tr>
<tr>
<td>Red</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>BUV395</td>
<td></td>
</tr>
</tbody>
</table>
PANEL DESIGN
Requisites for successful multicolor applications

A. Careful reagent selection

B. Optimal sample preparation and staining conditions

C. Proper cytometer performance, setup, and data collection

D. Proper data analysis
Typical challenges

• Some markers are highly expressed, others are expressed at low levels.

• Some dyes are much brighter than others.

• Significant emission spillover from non-primary fluorescent reagents contributes to optical background, which can often diminish the resolution of dim markers (due to spread after compensation).

• Some markers may be available only in certain colors.
Principles of panel design

1. Check for reagent availability/clone selection.

2. Match fluorochromes by brightness (values from stain index) according to antigen density and distribution (published values or TDS).


4. Run appropriate controls.
Antigen/fluorochrome combos (1)

- Classify the antigens you would like to measure

  **Primary:** Well characterized, easily classified positive or negative (CD45, CD3, CD4, etc).

  **Secondary:** Well characterized, also expressed at a higher density, often over a continuum (CD27, CD28, CD45RA/RO).

  **Tertiary:** Expressed at low levels only (CD25), also uncharacterized antigens.

- Use the brighter fluorochromes for dimly expressed markers.
- Use the dimmer fluorochromes for more highly expressed markers.

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Antigen/fluorochrome combos (2)

Antigen Group → Tertiary → Secondary → Primary

**Antigen Group**
- **BRIGHT**
  - BV421, BV650, BV711, BV786
  - PE, PE-CF594, PE-Cy7
  - APC

**Tertiary**
- **MODERATE**
  - BUV395
  - BV605, BV510
  - FITC, Alexa Fluor® 488, PerCP-Cy5.5
  - Alexa Fluor® 647, Alexa Fluor® 700

**Secondary**
- **DIM**
  - V450, V500, AmCyan
  - APC-Cy7/APC-H7

**Primary**

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Spillover

• Characterize spillovers in your own instrument (differences if using blue vs yellow-green laser, filter set, etc.)

• Think in terms of spillover categories:
  – Fluors excited by the same laser
  – Cross-laser (fluor excited by more than one laser)
  – Tandem dyes into primary detector (eg, PE-Cy7 into PE)
## Example spillover matrix: 14 colors

<table>
<thead>
<tr>
<th>Detector</th>
<th>Fluorochrome (spillover into)</th>
<th>BUV395</th>
<th>BV421</th>
<th>BV510</th>
<th>BV605</th>
<th>BV711</th>
<th>BV786</th>
<th>FITC</th>
<th>PerCP-Cy5.5</th>
<th>PE</th>
<th>PE-CF594</th>
<th>PE-Cy7</th>
<th>APC</th>
<th>Alexa 700</th>
<th>APC-H7</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV</td>
<td>BUV395</td>
<td>0.2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
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<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Violet</td>
<td>BV421</td>
<td>0.6</td>
<td>4.1</td>
<td>3.8</td>
<td>2.5</td>
<td>4.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>BV510</td>
<td>0.0</td>
<td>20.4</td>
<td>0.9</td>
<td>1.1</td>
<td>1.9</td>
<td>5.2</td>
<td>0.0</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>BV605</td>
<td>0.1</td>
<td>1.0</td>
<td>12.0</td>
<td>0.1</td>
<td>0.3</td>
<td>0.6</td>
<td>0.0</td>
<td>2.5</td>
<td>12.0</td>
<td>0.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td></td>
<td>BV711</td>
<td>0.0</td>
<td>0.1</td>
<td>1.7</td>
<td>24.6</td>
<td>2.6</td>
<td>0.1</td>
<td>75.0</td>
<td>0.4</td>
<td>3.4</td>
<td>0.1</td>
<td>1.0</td>
<td>7.0</td>
<td>0.2</td>
<td>0.2</td>
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<td></td>
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<td>0.1</td>
<td>0.0</td>
<td>0.3</td>
<td>4.7</td>
<td>27.0</td>
<td>0.0</td>
<td>10.0</td>
<td>0.0</td>
<td>0.6</td>
<td>0.6</td>
<td>2.2</td>
<td>11.0</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>Blue</td>
<td>FITC</td>
<td>0.1</td>
<td>0.0</td>
<td>4.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.8</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td></td>
<td>PerCP-Cy5.5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.2</td>
<td>3.4</td>
<td>0.0</td>
<td>0.0</td>
<td>3.5</td>
<td>7.9</td>
<td>64.0</td>
<td>0.2</td>
<td>1.8</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
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<tr>
<td>Yellow-Green</td>
<td>PE</td>
<td>2.0</td>
<td>0.1</td>
<td>0.4</td>
<td>10.9</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>1.0</td>
<td>28.0</td>
<td>2.1</td>
<td>0.4</td>
<td>0.5</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>PE-CF594</td>
<td>0.5</td>
<td>0.0</td>
<td>0.1</td>
<td>16.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>3.0</td>
<td>19.2</td>
<td>0.5</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
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<tr>
<td></td>
<td>PE-Cy7</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>2.1</td>
<td>1.4</td>
<td>2.2</td>
<td>0.0</td>
<td>14.7</td>
<td>1.0</td>
<td>11.6</td>
<td>3.1</td>
<td>5.5</td>
<td>52.3</td>
<td>52.3</td>
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<tr>
<td>Red</td>
<td>APC</td>
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<td>0.0</td>
<td>0.0</td>
<td>0.2</td>
<td>1.3</td>
<td>0.1</td>
<td>0.0</td>
<td>20.4</td>
<td>0.0</td>
<td>0.4</td>
<td>0.0</td>
<td>0.0</td>
<td>2.9</td>
<td>5.3</td>
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<tr>
<td></td>
<td>Alexa 700</td>
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<td>0.0</td>
<td>0.0</td>
<td>0.1</td>
<td>24.0</td>
<td>1.0</td>
<td>0.0</td>
<td>22.0</td>
<td>0.0</td>
<td>0.1</td>
<td>0.4</td>
<td>34.4</td>
<td>34.4</td>
<td>10.6</td>
</tr>
<tr>
<td></td>
<td>APC-H7</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>4.0</td>
<td>4.8</td>
<td>0.0</td>
<td>4.4</td>
<td>0.0</td>
<td>0.0</td>
<td>2.5</td>
<td>4.8</td>
<td>13.9</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spillover is</th>
<th>&lt; 0.5%</th>
<th>&lt; 3%</th>
<th>&lt; 10%</th>
<th>&lt; 20%</th>
<th>&lt; 30%</th>
<th>&gt; 30%</th>
</tr>
</thead>
</table>
5 FLUOROCHROMES, MINIMAL COMPENSATION
5 fluors, minimal compensation

- We have now developed 5 fluorochromes with:
  - Moderate-to-high brightness
  - Minimal or NO spillover between them

- Prerequisite: 5-laser flow cytometer

<table>
<thead>
<tr>
<th>Channel</th>
<th>BUV395</th>
<th>BV421</th>
<th>FITC</th>
<th>PE-CF594</th>
<th>APC</th>
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<tr>
<td>BUV395</td>
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<td>0.00</td>
<td>0.32</td>
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<tr>
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<tr>
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<td>0.11</td>
<td>0.23</td>
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<tr>
<td>PE-CF594</td>
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<td>0.04</td>
<td>0.00</td>
<td>0.16</td>
<td>0.00</td>
</tr>
<tr>
<td>APC</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Example: 5-color T-cell panel

- CD4 BUV395
- CD3 BV421
- CD8 FITC
- CD27 PE-CF594
- CD45RA APC
No compensation, optimal resolution

**Single-stained**
- CD4 BUV395: SI: 72
- CD3 BV421: SI: 129
- CD8 FITC: SI: 201
- CD45RA APC
- CD27 PE-CF594: SI: 32
- CD45RA APC

**Mixed**
- CD3 BV421: SI: 65
- CD4 BUV395: SI: 127
- CD8 FITC: SI: 200
- CD27 PE-CF594: SI: 33
- CD45RA APC: SI: 84
5-color B-cell panel

CD19 BUV395
CD27 BV421
IgD FITC
CD38 PE-CF594
IgM APC
14-COLOR LEUCOCYTES SUBSETTING PANEL
Panel description

• Panel aimed at identifying major leukocyte subsets in human peripheral blood:
  – T cells: CD3, CD4, CD8
  – Activated T cells: HLA-DR
  – Regulatory T cells: CD4, CD25
  – B cells: CD19, CD20
  – NK T cells: CD3, CD8, CD56, CD57
  – NK cells: CD56, CD16, CD8, CD57
  – DCs: HLA-DR, CD11c, CD123
  – Monocytes: CD14, CD33, HLA-DR, CD16
## Panel Design

<table>
<thead>
<tr>
<th>Antigen expression</th>
<th>Co-expression</th>
<th>Fluor assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>CD3</td>
<td>CD3 PerCP-Cy5.5</td>
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<tr>
<td>CD4</td>
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<td>CD4 BUV395</td>
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<tr>
<td>CD8</td>
<td>CD8</td>
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<td>CD57 FITC</td>
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<td>CD19</td>
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<td>CD20</td>
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<td>HLA-DR APC-H7</td>
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<tr>
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<td>CD14 V500</td>
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<tr>
<td>CD33</td>
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<td>CD33 PE-Cy7</td>
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<tr>
<td>CD25</td>
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<tr>
<td>CD123</td>
<td>CD123</td>
<td>CD123 BV421</td>
</tr>
<tr>
<td>CD11c</td>
<td>CD11c</td>
<td>CD11c PE</td>
</tr>
</tbody>
</table>
T cell/NK-T cell subsets

- CD3 PerCP-Cy5.5
- CD8 BV711
- CD4 T
- CD56 APC
- Tregs
- Activated Tregs
- HLA-DR APC-H7

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B cell/NK cell/Dendritic cell subsets

- CD57 APC
- CD20 AF700
- CD3 PerCP-Cy5.5
- CD56 APC
- CD11c PE
- CD123 BV421
- CD16 PE-CF594
- CD8 BV711
- CD19 BV786
- HLA-DR APC-H7
- pDC
- mDC

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Monocyte subsets

SSC vs FSC

CD14 vs CD500

CD33 PE-Cy7

HLA-DR APC-H7

CD16 PE-CF594

Pro-inflammatory

Monocytes
14-Color analysis
T/B/NK/NK-T/Mono/Dendritic cell subsets

- HLA-DR APC-H7
  - Pro-inflammatory
  - CD16 PE-CF594
  - CD14 V500
  - CD20 AF700
  - HLA-DR APC-H7
  - CD19 BV786

- Monocytes
  - Monocytes
  - CD33 PE-Cy7
  - CD4 T
  - NK

- Non-B, Non-T
  - CD57 APC
  - B cell
  - T cell
  - NK

- Dendritic Cell
  - pDC
  - mDC
  - CD123 BV421

- NK
  - CD57 FITC
  - CD56 APC
  - CD8 BV711
  - CD16 PE-CF594
  - CD25 BV605
  - T regs
  - Activated T regs

- CD4 T
  - CD4 BUV395
  - CD4 BUV395
  - HLA-DR APC-H7

BD
Helping all people live healthy lives
13-COLOR T-CELL PANEL
## Panel design

<table>
<thead>
<tr>
<th>Antigen expression</th>
<th>Co-expression</th>
<th>Fluor assignment</th>
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<tbody>
<tr>
<td>Viability</td>
<td>Viability</td>
<td>Viability (V500)</td>
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<td>*CD4</td>
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<td>*CCR7 BV421</td>
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<tr>
<td>CXCR3</td>
<td>CXCR3</td>
<td>CXCR3 Alexa Fluor® 700</td>
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</tbody>
</table>

Markers of the same color are co-expressed. Asterisked markers co-express with their same color and also with markers of their opposite color (either green or purple).
Memory/effector/activated subsets
Tregs/Th1/Th2/Th17

CD8 FITC

CD4 FITC

CD4 BUV395

CD127 APC

CD25 PE-CF594

TReg

Naive

Memory

Memory Activated

HLA-DR APC-H7

CD45RO PE-Cy7

T Reg

T H17

T H2

T H1

CXCR3

Alexa Fluor® 700

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REPLACING QDOT® REAGENTS WITH BV REAGENTS IN A 12-COLOR PANEL
### Table 2. Reagents used for OMIP-013

<table>
<thead>
<tr>
<th>SPECIFICITY</th>
<th>CLONE</th>
<th>FLUOROCHROME</th>
<th>PURPOSE</th>
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<td>CD3</td>
<td>SK7</td>
<td>APC-H7</td>
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<td>RPA-T8</td>
<td>QD585</td>
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<td>150503</td>
<td>Ax680</td>
<td>memory/</td>
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<tr>
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<td>O323</td>
<td>FITC</td>
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<td>PE-Cy5.5</td>
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<td>Dead cells</td>
<td>–</td>
<td>AqBlu</td>
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</tr>
</tbody>
</table>

Original panel (2)
Proposed alternative panel

- CD57 FITC
- CD28 PerCP-Cy5.5
- CD31 PE
- CCR7 PE-CF594
- CD95 PE-Cy7
- CD62L APC
- CD45RA Alexa Fluor® 700
- CD3 APC-H7
- CD127 BV421
- CD4 BV510
- CD27 BV605
- CD8 BV711
Results: CD8 subsets
Results: CD4 subsets
Conclusions

• BV dyes offer a wide array of options for successful multicolor flow cytometry

• Brightness is a key feature of BV dyes that enhances resolution

• Panel design needs to be carefully assessed in order to mitigate spillover issues

• BUV dyes are an exciting and promising new family of dyes
Acknowledgements

- Alan Stall
- Brent Gaylord
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- Cynthia Lane
- Kimberly Duffy