

# ARIA

|                                  |                 | Secondary Fluochrome (Bright Antigen) |          |       |       |       |       |       |       |      |        |       |     |          |        |             |       |        |     |        |        |          |         |          |               |     |
|----------------------------------|-----------------|---------------------------------------|----------|-------|-------|-------|-------|-------|-------|------|--------|-------|-----|----------|--------|-------------|-------|--------|-----|--------|--------|----------|---------|----------|---------------|-----|
|                                  |                 | BV421                                 | Pac Blue | BV480 | BV510 | BV605 | BV650 | BV711 | BV786 | FITC | AF 488 | BB515 | PE  | PE-CF594 | PE-Cy5 | PerCP-Cy5.5 | BB700 | PE-Cy7 | APC | AF 647 | AF 700 | APC-R700 | APC-Cy7 | APC-Fire | APC-eFluor780 |     |
| Primary Fluochrome (Dim Antigen) | BV421           | 1813                                  |          | 42%   | 42%   | 77%   | 77%   | 80%   | 81%   | 26%  | 39%    | 35%   | 51% | 51%      | 50%    | 49%         | 67%   | 55%    | 42% | 39%    | 45%    | 51%      | 34%     | 51%      | 52%           |     |
|                                  | Pacific Blue    |                                       | 69       | 42%   | 42%   | 77%   | 77%   | 80%   | 81%   | 26%  | 39%    | 35%   | 51% | 51%      | 50%    | 49%         | 67%   | 55%    | 42% | 39%    | 45%    | 51%      | 34%     | 51%      | 52%           |     |
|                                  | BV480           | 54%                                   | 38%      | 310   |       | 25%   | 22%   | 26%   | 26%   | 34%  | 39%    | 37%   | 45% | 41%      | 26%    | 27%         | 29%   | 38%    | 32% | 35%    | 44%    | 40%      | 22%     | 34%      | 45%           |     |
|                                  | BV510           | 54%                                   | 38%      |       | 158   | 25%   | 22%   | 26%   | 26%   | 34%  | 39%    | 37%   | 45% | 41%      | 26%    | 27%         | 29%   | 38%    | 32% | 35%    | 44%    | 40%      | 22%     | 34%      | 45%           |     |
|                                  | BV605           | 43%                                   | 32%      | 83%   | 86%   | 599   | 78%   | 20%   | 20%   | 26%  | 15%    | 41%   | 87% | 89%      | 50%    | 7%          | 60%   | 26%    | 31% | 10%    | 31%    | 26%      | 22%     | 34%      | 27%           |     |
|                                  | BV650           | 37%                                   | 18%      | 81%   | 85%   | 94%   | 451   | 61%   | 33%   | 17%  | 12%    | 17%   | 79% | 84%      | 92%    | 58%         | 84%   | 13%    | 86% | 23%    | 17%    | 42%      | 21%     | 21%      | 39%           |     |
|                                  | BV711           | 27%                                   | 15%      | 71%   | 81%   | 92%   | 94%   | 612   | 78%   | 13%  | 21%    | 31%   | 77% | 85%      | 90%    | 88%         | 96%   | 41%    | 81% | 43%    | 70%    | 87%      | 24%     | 26%      | 33%           |     |
|                                  | BV786           | 25%                                   | 9%       | 64%   | 77%   | 90%   | 92%   | 96%   | 656   | 12%  | 12%    | 7%    | 59% | 76%      | 89%    | 85%         | 94%   | 93%    | 77% | 41%    | 63%    | 84%      | 80%     | 85%      | 85%           |     |
|                                  | FITC            | 21%                                   | 31%      | 62%   | 30%   | 25%   | 25%   | 30%   | 30%   | 38   |        |       | 52% | 39%      | 36%    | 37%         | 45%   | 38%    | 36% | 41%    | 41%    | 41%      | 23%     | 43%      | 41%           |     |
|                                  | Alexa Fluor 488 | 21%                                   | 31%      | 62%   | 30%   | 25%   | 25%   | 30%   | 30%   |      | 55     |       | 52% | 39%      | 36%    | 37%         | 45%   | 38%    | 36% | 41%    | 41%    | 41%      | 23%     | 43%      | 41%           |     |
|                                  | BB515           | 21%                                   | 31%      | 62%   | 30%   | 25%   | 25%   | 30%   | 30%   |      |        | 547   | 52% | 39%      | 36%    | 37%         | 45%   | 38%    | 36% | 41%    | 41%    | 41%      | 23%     | 43%      | 41%           |     |
|                                  | PE              | 13%                                   | 15%      | 37%   | 16%   | 57%   | 18%   | 22%   | 18%   | 53%  | 58%    | 81%   | 555 | 85%      | 54%    | 22%         | 56%   | 43%    | 22% | 21%    | 24%    | 30%      | 13%     | 29%      | 28%           |     |
|                                  | PE-CF594        | 6%                                    | 8%       | 40%   | 13%   | 77%   | 33%   | 20%   | 10%   | 52%  | 55%    | 81%   | 93% | 349      | 69%    | 21%         | 75%   | 45%    | 12% | 13%    | 15%    | 15%      | 8%      | 12%      | 15%           |     |
|                                  | PE-Cy5          | 0%                                    | 2%       | 24%   | 3%    | 75%   | 54%   | 82%   | 1%    | 39%  | 37%    | 68%   | 90% | 93%      | 691    |             | 33%   | 51%    | 24% | 16%    | 60%    | 0%       | 2%      | 5%       |               |     |
|                                  | PerCP-Cy5.5     | 0%                                    | 2%       | 24%   | 3%    | 75%   | 54%   | 82%   | 1%    | 39%  | 37%    | 68%   | 90% | 93%      |        | 88          |       | 33%    | 51% | 24%    | 16%    | 60%      | 0%      | 2%       | 5%            |     |
|                                  | BB700           | 0%                                    | 2%       | 24%   | 3%    | 75%   | 54%   | 82%   | 1%    | 39%  | 37%    | 68%   | 90% | 93%      |        |             | 564   | 33%    | 51% | 24%    | 16%    | 60%      | 0%      | 2%       | 5%            |     |
|                                  | PE-Cy7          | 0%                                    | 0%       | 1%    | 0%    | 63%   | 35%   | 85%   | 81%   | 20%  | 17%    | 44%   | 81% | 89%      | 95%    | 88%         | 96%   | 358    | 38% | 6%     | 11%    | 60%      | 38%     | 52%      | 53%           |     |
|                                  | APC             | 13%                                   | 8%       | 43%   | 50%   | 79%   | 92%   | 62%   | 31%   | 25%  | 15%    | 17%   | 45% | 63%      | 96%    | 65%         | 83%   | 85%    | 30% | 414    |        | 33%      | 80%     | 57%      | 60%           | 77% |
|                                  | Alexa Fluor 647 | 13%                                   | 8%       | 43%   | 50%   | 79%   | 92%   | 62%   | 31%   | 25%  | 15%    | 17%   | 45% | 63%      | 96%    | 65%         | 83%   | 85%    | 30% |        | 96     | 33%      | 80%     | 57%      | 60%           | 77% |
|                                  | Alexa Fluor 700 | 12%                                   | 15%      | 43%   | 61%   | 82%   | 92%   | 96%   | 60%   | 12%  | 17%    | 22%   | 65% | 78%      | 94%    | 87%         | 95%   | 39%    | 94% | 91%    |        | 34       | 60%     | 64%      | 71%           |     |
| APC-R700                         | 12%             | 15%                                   | 43%      | 61%   | 82%   | 92%   | 96%   | 60%   | 12%   | 17%  | 22%    | 65%   | 77% | 94%      | 87%    | 95%         | 39%   | 94%    | 91% |        |        | 429      | 60%     | 64%      | 71%           |     |
| APC-Cy7                          | 3%              | 4%                                    | 1%       | 2%    | 22%   | 79%   | 91%   | 85%   | 8%    | 1%   | 1%     | 6%    | 30% | 89%      | 74%    | 91%         | 84%   | 88%    | 81% | 77%    | 92%    | 66       |         |          |               |     |
| APC-Fire                         | 3%              | 4%                                    | 1%       | 2%    | 22%   | 79%   | 91%   | 85%   | 8%    | 1%   | 1%     | 6%    | 30% | 89%      | 74%    | 91%         | 84%   | 88%    | 81% | 77%    | 92%    |          | 109     |          |               |     |
| APC-eFluor780                    | 3%              | 4%                                    | 1%       | 2%    | 22%   | 79%   | 91%   | 85%   | 8%    | 1%   | 1%     | 6%    | 30% | 89%      | 74%    | 91%         | 84%   | 88%    | 81% | 77%    | 92%    |          |         | 115      |               |     |

### How the matrix was made

Mouse spleens was stained with individual anti-CD8 labeled antibodies with the indicated fluochrome and analyzed on the indicated instrument.

Each calculated value was arbitrarily assigned a color code according to the legend to show where the biggest spreading was situated.

### How to use the resolution impact matrix

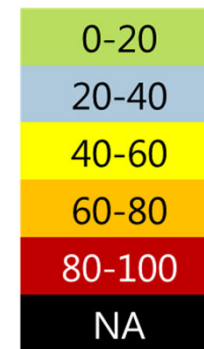
You find the color of interest on the top of the matrix, go down till you find the channel that you need to combine the color with and read the impact of spreading.

### Consider this for panel design

When you are designing larger panels the task of making correct combinations becomes more difficult, but using the list below can help you:

- The lineage markers such as CD4, CD19 etc. should be found on the top of the matrix.
- Make sure the lineage marker has as many green cells as possible.
- For an important marker you should find the color on the left of the matrix.
- Make sure the marker has as many green cells as possible when you move across the matrix.
- Notice that spreading occurs between different laser lines.

### Amount of spreading (%)



### Relative fluochrome brightness (AU)

