Fortessa 5

Flow Cytometry & Single Cell Analysis
https://fcsc.ku.dk/

Primary Flourochrome (Dim Antigen) \ Secondary Flourochrome (Bright Antigen)	BUV395	BUV496	BUV737	BV421	Pacific Blu	BV480	BV510	BV605	BV650	BV711	BV786	FITC	Alexa Fluo	BB515	PerCP-Cy5	BB700	PE	PE-CF594	PE-Cy5	PE-Cy7	APC	Alexa Fluo	Alexa Fluo	APC-R700	APC-Cy7	APC-Fire	APC-eFlou
BUV395	112	26%	36%	11%	20%	13%	10%	10%	15%	15%	13%	14%	13%	14%	18%	11%	11%	12%	31%	16%	16%	13%	18%	13%	12%	9%	15%
BUV496	38%	97	31%	31%	16%	60%	51%	21%	24%	25%	22%	27%	15%	33%	30%	4%	22%	23%	42%	27%	24%	4%	30%	7%	29%	9%	29%
BUV737	8%	29%	407	9%	11%	15%	31%	67%	62%	68%	65%	9%	10%	8%	57%	69%	21%	35%	76%	44%	53%	15%	28%	60%	26%	27%	27%
BV421	27%	26%	25%	305		26%	29%	30%	45%	40%	64%	29%	36%	29%	32%	27%	23%	25%	31%	29%	25%	27%	31%	27%	29%	29%	29%
Pacific Blu	26%	26%	25%		7	26%	29%	29%	44%	38%	62%	29%	36%	29%	32%	28%	23%	25%	31%	29%	25%	26%	31%	28%	29%	29%	29%
BV480	30%	40%	28%	32%	23%	47		27%	29%	30%	30%	32%	19%	33%	35%	8%	24%	26%	42%	32%	27%	8%	33%	9%	33%	8%	33%
BV510	30%	40%	28%	31%	22%		19	27%	29%	30%	30%	32%	18%	33%	35%	7%	24%	26%	42%	32%	27%	7%	33%	8%	33%	8%	33%
BV605	17%	26%	14%	20%	0%	49%	49%	134	49%	19%	17%	20%	0%	18%	20%	12%	45%	53%	20%	18%	18%	0%	19%	0%	18%	0%	19%
BV650	6%	17%	13%	14%	11%	44%	50%	83%	94	17%	12%	9%	8%	7%	37%	47%	41%	51%	86%	9%	56%	9%	8%	17%	6%	13%	13%
BV711	9%	14%	63%	11%	0%	31%	33%	77%	78%	46	31%	10%	0%	0%	67%	76%	53%	70%	92%	19%	54%	0%	22%	44%	10%	0%	0%
BV786	9%	12%	73%	9%	9%	27%	39%	75%	76%	79%	294	13%	6%	8%	66%	78%	22%	39%	70%	76%	42%	10%	24%	53%	60%	56%	56%
FITC	28%	31%	27%	24%	34%	32%	26%	23%	25%	26%	22%	17			30%	30%	24%	22%	42%	27%	23%	23%	29%	24%	28%	23%	25%
Alexa Fluo	28%	31%	27%	24%	34%	32%	26%	23%	25%	26%	22%		34		30%	30%	24%	22%	42%	27%	24%	23%	29%	24%	28%	23%	25%
BB515	28%	32%	27%	24%	34%	33%	26%	23%	25%	26%	22%			88	30%	31%	24%	22%	43%	27%	24%	23%	29%	24%	28%	23%	25%
PerCP-Cy5	15%	17%	71%	13%	0%	16%	0%	50%	34%	39%	15%	21%	1%	3%	26		65%	79%	96%	31%	59%	5%	24%	26%	20%	0%	3%
BB700	15%	17%	71%	13%	0%	16%	0%	50%	34%	39%	15%	21%	1%	4%		173	65%	79%	96%	31%	59%	6%	24%	26%	20%	0%	4%
PE	9%	9%	12%	7%	13%	13%	13%	55%	13%	20%	6%	15%	4%	12%	9%	6%	623	65%	95%	53%	11%	10%	13%	9%	0%	4%	11%
PE-CF594	11%	13%	15%	13%	18%	31%	35%	83%	33%	17%	9%	16%	21%	12%	11%	19%	88%	362	55%	33%	15%	17%	17%	17%	4%	19%	12%
PE-Cy5	5%	5%	35%	3%	0%	6%	0%	63%	42%	11%	3%	7%	0%	0%	53%	7%	69%	79%	351	16%	75%	25%	8%	20%	24%	0%	9%
PE-Cy7	7%	8%	71%	5%	8%	11%	11%	61%	39%	32%	49%	10%	8%	11%	62%	60%	59%	77%	94%	465	67%	47%	38%	74%	79%	77%	78%
APC	3%	7%	39%	5%	0%	12%	7%	35%	59%	17%	14%	7%	0%	5%	48%	64%	36%	52%	97%	20%	185		16%	68%	81%	41%	66%
Alexa Fluo	3%	7%	39%	5%	0%	12%	7%	35%	59%	17%	14%	7%	0%	5%	48%	64%	36%	52%	97%	20%		70	16%	68%	81%	41%	66%
Alexa Fluo	8%	11%	92%	7%	7%	13%	14%	36%	58%	66%	30%	11%	7%	8%	62%	82%	14%	26%	92%	25%	86%	80%	19		58%	45%	52%
APC-R700	8%	11%	92%	7%	7%	13%	13%	36%	58%	66%	30%	11%	7%	8%	63%	82%	13%	26%	92%	25%	86%	80%		185	59%	45%	53%
APC-Cy7	4%	4%	82%	2%	1%	7%	0%	7%	32%	50%	63%	4%	0%	1%	48%	72%	4%	7%	79%	72%	75%	64%	53%	81%	70		
APC-Fire	4%	4%	82%	2%	1%	7%	0%	7%	32%	50%	63%	4%	0%	1%	48%	72%	4%	7%	79%	72%	75%	64%	53%	81%		69	
APC-eFlou	4%	4%	82%	2%	1%	7%	0%	7%	32%	50%	63%	4%	0%	1%	48%	72%	4%	7%	79%	72%	75%	64%	53%	81%			72

How the matrix was made
Mouse spleens was stained with individual anti-CD8 labeled antibodies with the indicated flourochrome 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 (%)

0-20
20-40
40-60
60-80
80-100
NA

Relative fluorochrome brightness (AU)

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