Fortessa X20

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

Primary Flourochrome (Dim Antigen)	Secondary Flourochrome (Bright Antigen)	BUV395	BUV496	BUV737	BV421	Pac Blue	BV480	BV510	BV605	BV650	BV711	BV786	FITC	AF 488	BB515	PerCP-Cy5.5	BB700	PE	PE-CF594	PE-Cy5	PE-Cy7	APC	AF 647	AF 700	APC-R700	APC-Cy7	APC-Fire	APC-eFlour780
	BUV395	154	48%	47%	5%	9%	11%	8%	6%	8%	7%	8%	7%	5%	6%	9%	8%	8%	6%	14%	6%	4%	7%	8%	7%	10%	7%	8%
	BUV496	68%	157	33%	49%	22%	83%	76%	18%	16%	14%	16%	23%	15%	39%	21%	16%	16%	17%	29%	19%	20%	12%	19%	13%	26%	17%	23%
	BUV737	6%	27%	331	4%	2%	16%	34%	68%	66%	71%	66%	6%	4%	4%	62%	73%	23%	41%	75%	47%	59%	17%	35%	68%	38%	30%	30%
	BV421	16%	16%	22%	510		19%	20%	46%	48%	45%	67%	18%	17%	22%	26%	34%	21%	26%	63%	20%	20%	20%	24%	25%	29%	26%	27%
	Pacific Blue	15%	16%	22%		12	19%	19%	45%	47%	45%	67%	17%	16%	21%	26%	35%	21%	27%	63%	20%	20%	19%	24%	25%	29%	26%	27%
	BV480	31%	66%	29%	44%	26%	106		29%	32%	30%	29%	35%	23%	38%	39%	31%	32%	32%	43%	33%	32%	22%	42%	30%	43%	31%	43%
	BV510	31%	66%	29%	43%	26%		41	29%	31%	30%	29%	35%	23%	38%	39%	30%	32%	32%	43%	33%	32%	21%	42%	30%	43%	31%	43%
	BV605	11%	25%	22%	21%	8%	60%	61%	164	66%	18%	14%	17%	2%	14%	34%	37%	59%	66%	73%	18%	33%	3%	22%	4%	22%	3%	22%
	BV650	5%	18%	65%	10%	4%	47%	53%	85%	79	39%	16%	10%	0%	9%	69%	70%	53%	68%	93%	21%	71%	7%	11%	22%	18%	5%	17%
	BV711	5%	14%	64%	10%	0%	40%	48%	81%	82%	43	40%	10%	0%	8%	72%	83%	52%	69%	91%	24%	60%	6%	25%	54%	19%	1%	14%
	BV786	3%	12%	73%	7%	5%	33%	49%	79%	79%	82%	306	10%	5%	5%	71%	84%	27%	45%	75%	80%	51%	17%	33%	60%	67%	65%	65%
	FITC	21%	34%	22%	19%	25%	39%	23%	20%	22%	21%	21%	31			27%	37%	30%	24%	39%	25%	26%	23%	32%	29%	31%	30%	31%
	Alexa Fluor 488	21%	34%	22%	19%	25%	39%	23%	20%	22%	21%	21%		50		27%	37%	31%	24%	39%	25%	26%	23%	32%	29%	31%	30%	31%
	BB515	21%	34%	22%	20%	25%	39%	23%	20%	22%	21%	21%			159	27%	37%	31%	24%	39%	25%	26%	23%	32%	29%	31%	30%	31%
	PerCP-Cy5.5	5%	5%	83%	2%	0%	9%	0%	57%	41%	48%	13%	12%	0%	17%	30		71%	84%	96%	46%	66%	7%	21%	38%	23%	0%	21%
	BB700	5%	5%	83%	2%	0%	9%	0%	58%	41%	49%	13%	12%	0%	17%		201	71%	84%	97%	47%	66%	8%	21%	38%	23%	0%	21%
	PE	18%	18%	20%	20%	21%	24%	28%	82%	29%	24%	17%	20%	18%	22%	21%	25%	553	84%	94%	54%	20%	21%	23%	23%	22%	23%	22%
	PE-CF594	10%	13%	14%	9%	11%	32%	36%	87%	44%	12%	7%	9%	9%	10%	14%	21%	92%	463	66%	37%	22%	12%	13%	15%	14%	15%	13%
	PE-Cy5	1%	1%	45%	0%	0%	3%	0%	65%	44%	12%	2%	5%	0%	0%	57%	14%	72%	85%	336	14%	77%	27%	13%	25%	25%	0%	21%
	PE-Cy7	2%	3%	69%	2%	7%	5%	6%	62%	41%	34%	50%	4%	6%	2%	65%	62%	61%	80%	95%	424	70%	54%	45%	78%	82%	80%	80%
	APC	0%	1%	34%	0%	0%	12%	3%	38%	61%	23%	10%	10%	0%	0%	55%	79%	35%	58%	97%	12%	169		24%	74%	60%	26%	53%
	Alexa Fluor 647	0%	1%	34%	0%	0%	12%	3%	38%	61%	23%	10%	10%	0%	0%	55%	79%	35%	58%	97%	12%		81	24%	74%	60%	26%	53%
	Alexa Fluor 700	0%	1%	86%	0%	6%	10%	16%	43%	61%	66%	31%	1%	4%	1%	63%	85%	9%	24%	90%	26%	88%	82%	22		58%	43%	50%
	APC-R700	0%	1%	87%	0%	6%	10%	15%	42%	61%	66%	31%	1%	4%	1%	63%	85%	9%	24%	90%	26%	88%	82%		185	58%	43%	50%
	APC-Cy7	0%	3%	80%	0%	1%	4%	0%	5%	34%	51%	65%	5%	2%	0%	49%	75%	2%	6%	79%	70%	77%	68%	58%	83%	79		
	APC-Fire	0%	3%	80%	0%	1%	4%	0%	5%	34%	51%	65%	5%	2%	0%	49%	75%	2%	6%	79%	70%	77%	68%	58%	83%		72	
	APC-eFlour780	0%	3%	80%	0%	1%	4%	0%	5%	34%	51%	65%	5%	2%	0%	49%	75%	2%	6%	79%	70%	77%	68%	58%	83%			74

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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