Fortessa 3

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

Primary Flourochrome (Dim Antigen)	Secondary Flourochrome (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-eFlour780
BV421	**347**		16%	13%	20%	42%	41%	55%	16%	17%	10%	13%	17%	12%	6%	24%	12%	14%	19%	16%	9%	10%	11%	14%
Pacific Blue		**13**	15%	13%	20%	42%	41%	55%	16%	16%	10%	13%	17%	12%	6%	24%	12%	14%	19%	16%	9%	10%	11%	14%
BV480	27%	8%	**64**		15%	12%	14%	18%	16%	0%	18%	15%	18%	12%	0%	27%	10%	14%	0%	16%	13%	13%	0%	17%
BV510	27%	7%		**31**	15%	12%	14%	18%	16%	0%	18%	15%	18%	12%	0%	27%	10%	14%	0%	16%	13%	13%	0%	17%
BV605	16%	0%	53%	53%	**157**	52%	7%	10%	11%	0%	5%	47%	59%	9%	0%	30%	7%	8%	0%	1%	6%	4%	0%	7%
BV650	0%	0%	50%	60%	83%	**156**	24%	2%	0%	0%	0%	42%	57%	74%	32%	60%	0%	59%	0%	0%	7%	4%	0%	3%
BV711	3%	0%	35%	52%	80%	83%	**291**	42%	0%	1%	0%	37%	59%	70%	72%	87%	4%	52%	13%	42%	68%	5%	2%	7%
BV786	2%	0%	22%	44%	76%	76%	90%	**289**	0%	0%	0%	15%	39%	61%	63%	83%	83%	41%	13%	30%	57%	67%	70%	69%
FITC	17%	21%	26%	10%	12%	6%	7%	9%	**33**			15%	14%	8%	3%	25%	3%	8%	12%	13%	8%	11%	11%	13%
Alexa Fluor 488	17%	21%	26%	10%	12%	6%	7%	9%		**42**		15%	14%	8%	3%	25%	3%	8%	12%	13%	8%	11%	11%	13%
BB515	18%	21%	26%	10%	12%	6%	7%	9%			**148**	15%	14%	8%	3%	25%	3%	8%	12%	13%	8%	11%	11%	13%
PE	8%	4%	4%	0%	5%	1%	1%	1%	17%	17%	37%	**171**	22%	25%	0%	19%	8%	0%	0%	0%	0%	0%	0%	0%
PE-CF594	1%	5%	15%	0%	40%	5%	0%	3%	27%	27%	45%	79%	**172**	17%	0%	36%	5%	0%	0%	0%	3%	0%	0%	0%
PE-Cy5	0%	1%	2%	0%	43%	24%	70%	0%	11%	11%	28%	74%	84%	**217**			12%	13%	6%	11%	43%	0%	0%	0%
PerCP-Cy5.5	0%	1%	2%	0%	43%	24%	70%	0%	11%	11%	28%	74%	84%		**46**		12%	13%	6%	11%	43%	0%	0%	0%
BB700	0%	1%	2%	0%	43%	24%	70%	0%	11%	11%	28%	74%	84%			**301**	12%	13%	6%	11%	43%	0%	0%	0%
PE-Cy7	0%	1%	4%	0%	39%	19%	73%	70%	2%	6%	15%	64%	80%	88%	80%	92%	**320**	14%	4%	11%	41%	41%	43%	41%
APC	0%	0%	0%	0%	2%	66%	32%	0%	20%	0%	0%	0%	12%	93%	54%	78%	0%	**181**		39%	71%	69%	34%	63%
Alexa Fluor 647	0%	0%	0%	0%	2%	66%	32%	0%	20%	0%	0%	0%	12%	93%	54%	78%	0%		**168**	39%	71%	69%	34%	63%
Alexa Fluor 700	0%	0%	0%	0%	20%	61%	85%	16%	0%	0%	0%	0%	24%	86%	61%	86%	16%	85%	85%	**32**		61%	45%	53%
APC-R700	0%	0%	0%	0%	20%	60%	85%	16%	0%	0%	0%	0%	23%	85%	61%	86%	16%	85%	85%		**196**	61%	45%	53%
APC-Cy7	0%	0%	0%	0%	0%	29%	68%	55%	0%	0%	0%	0%	0%	70%	40%	74%	70%	70%	68%	55%	81%	**63**		
APC-Fire	0%	0%	0%	0%	0%	29%	68%	55%	0%	0%	0%	0%	0%	70%	40%	74%	70%	70%	68%	55%	81%		**58**	
APC-eFlour780	0%	0%	0%	0%	0%	29%	68%	55%	0%	0%	0%	0%	0%	70%	40%	74%	70%	70%	68%	55%	81%			**62**

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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