Maximizing Conventional Cytometry with BD FACSymphony A5

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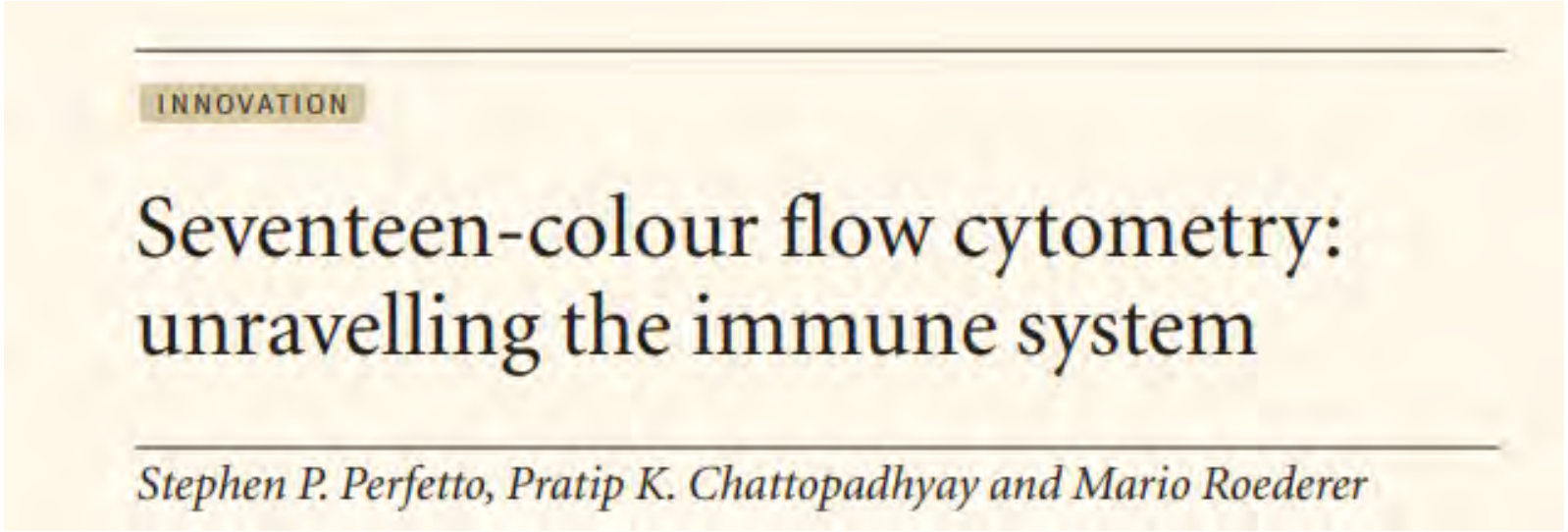
Fact: 17 color flow cytometry has existed for nearly 20 years

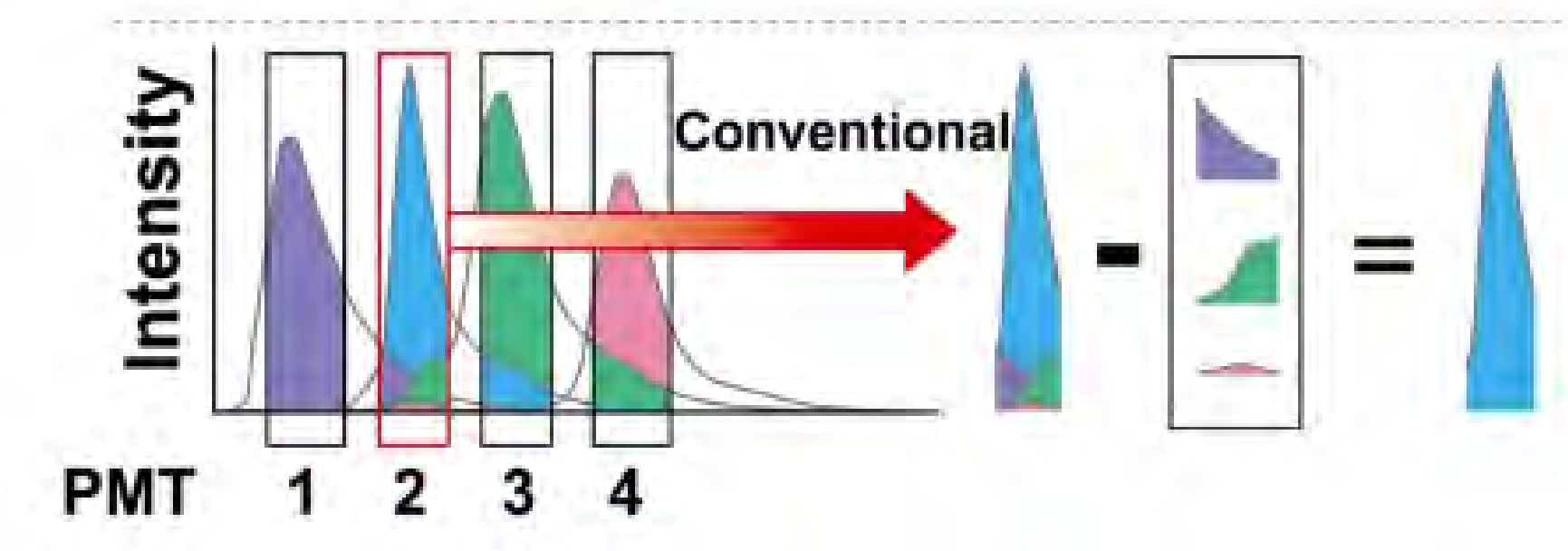


INNOVATION

Seventeen-colour flow cytometry: unravelling the immune system

Stephen P. Perfetto, Pratip K. Chattopadhyay and Mario Roederer

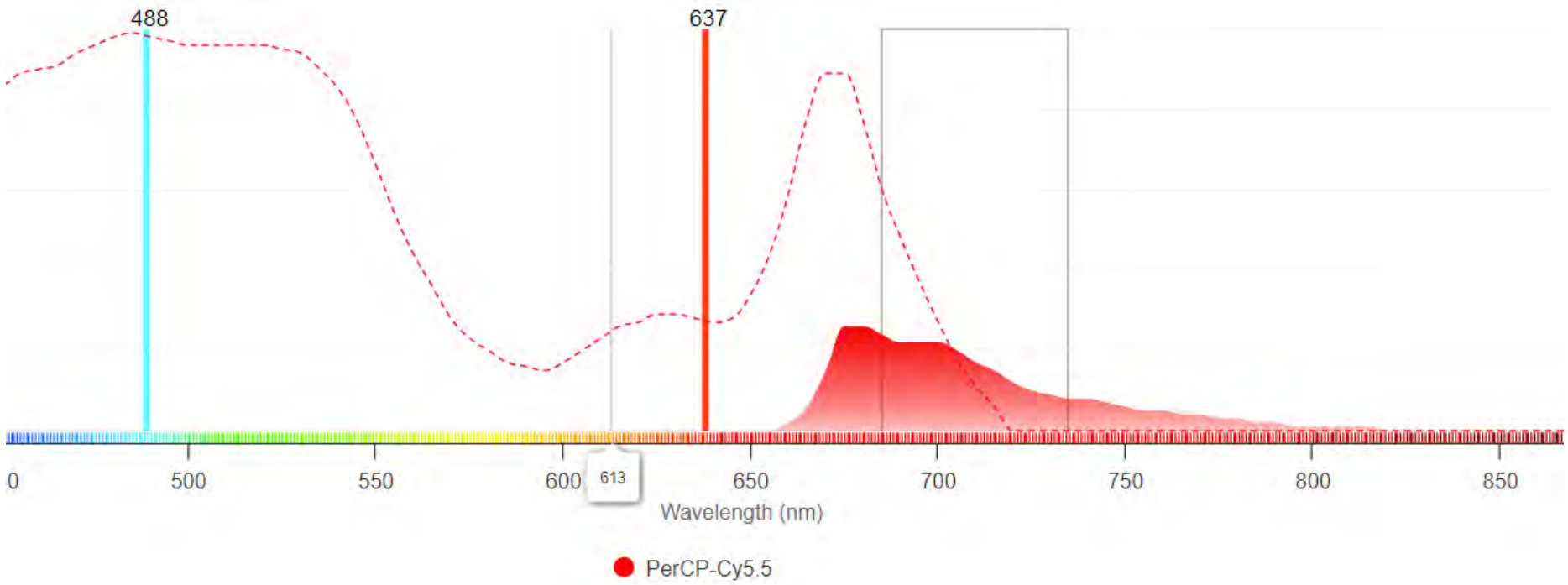
Refresher: Compensation



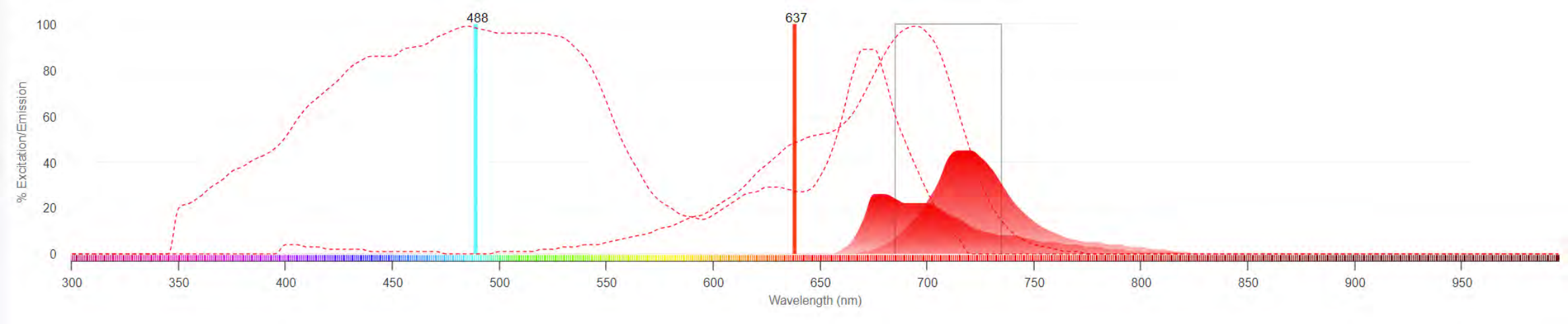
https://doi.org/10.1002/cyto.a.22725

Example : PerCP-Cy5.5 and AF700

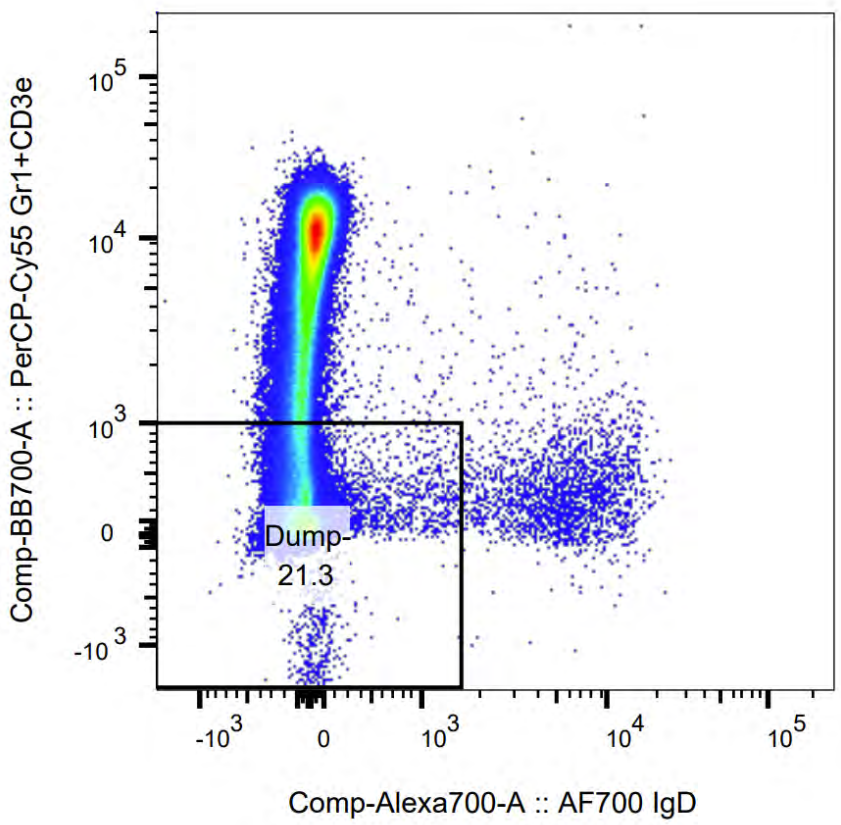
Filter sets for both on the A5: 710/50 (i.e. 685-735nm captured)



Example : PerCP-Cy5.5 and AF700



Example : PerCP-Cy5.5 and AF700



Why would I want to use a cytometer with 30 channels?

High Parameter Users:

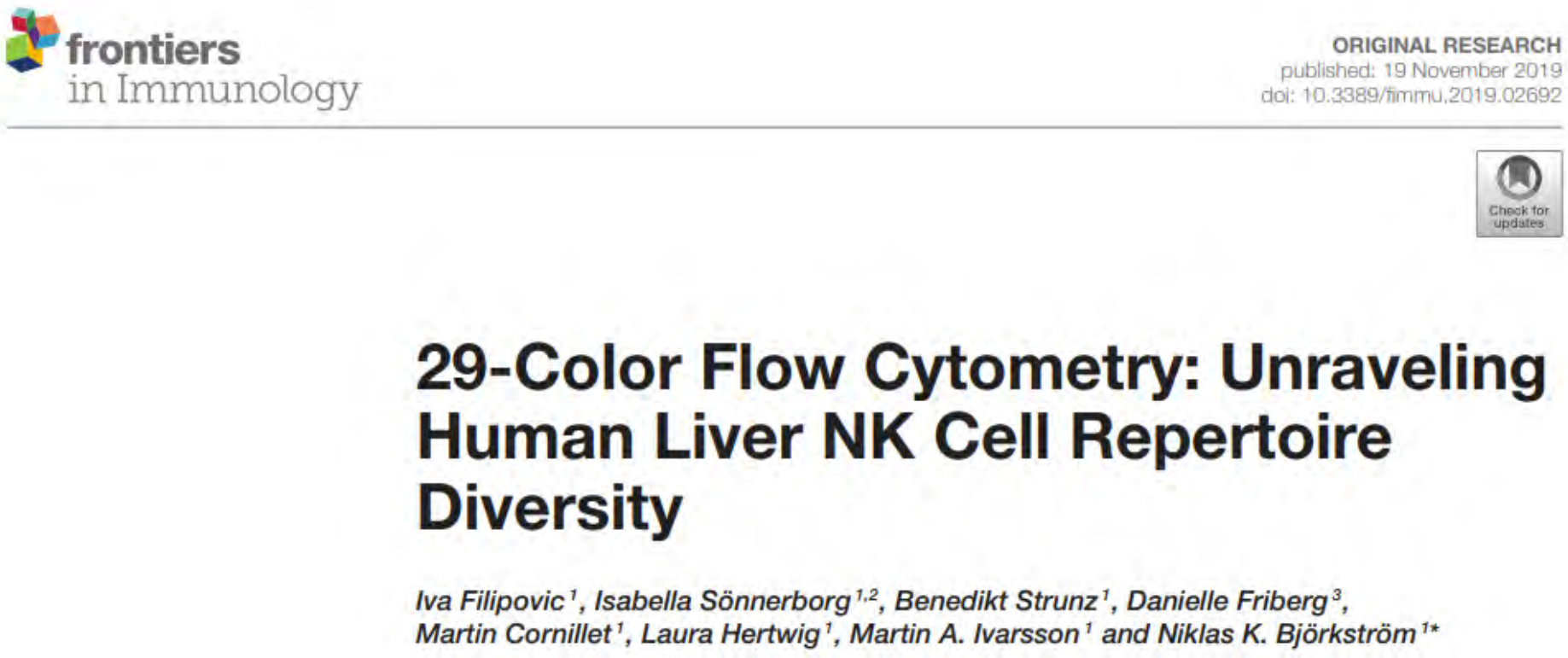
- LSRFortessa users looking to expand their panels who don't wish utilize spectral cytometry

Small Panel Users:

- Ease of panel design due to channel availability.

Everyone: Not a busy instrument at the moment.
Panel transfer to S6!

High parameter panels are possible with conventional flow cytometry



frontiers in Immunology

ORIGINAL RESEARCH
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Check for updates

29-Color Flow Cytometry: Unraveling Human Liver NK Cell Repertoire Diversity

*Iva Filipovic[1], Isabella Sönnerborg[1,2], Benedikt Strunz[1], Danielle Friberg[3], Martin Cornillet[1], Laura Hertwig[1], Martin A. Ivarsson[1] and Niklas K. Björkström[1]**

Conventional High Parameter Panel Design

- Traditional conventional design rules apply BUT we take advantage of new fluorophores with superior properties
- Unique fluorophores for both violet and UV lasers are well described and enable us to add several more parameters
- Choosing opposite ends of the spectrum if you know your gating scheme
- Titration Schemes are just as important!
  - Sometimes we will need to titrate secondary and tertiary markers with primary markers

Consequences of Poor Fluorophore Choice

Sometimes the fluorochrome listed by the manufacturer is not always the best choice in high parameter panels.

Examples:

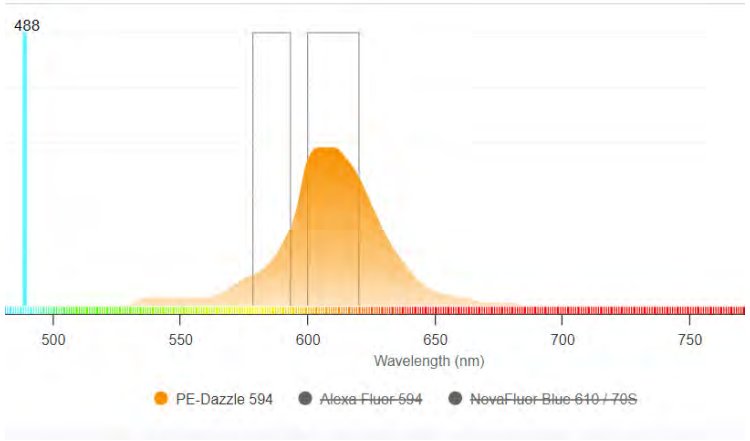
PE-dazzle594/CF594 tandems are in most cases not appropriate, AF594 is a more suitable alternative as it has less spillover into the PE channel and is not excited by the blue laser, thus opening another channel.

PerCP):

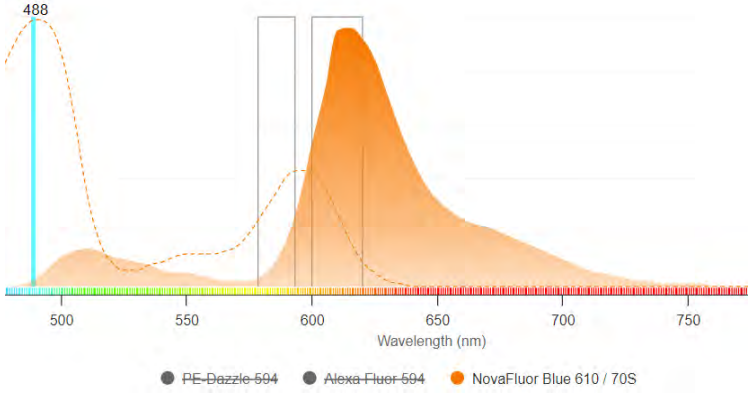
DAPI* and BV421):

Aqua viability dyes and BUV496

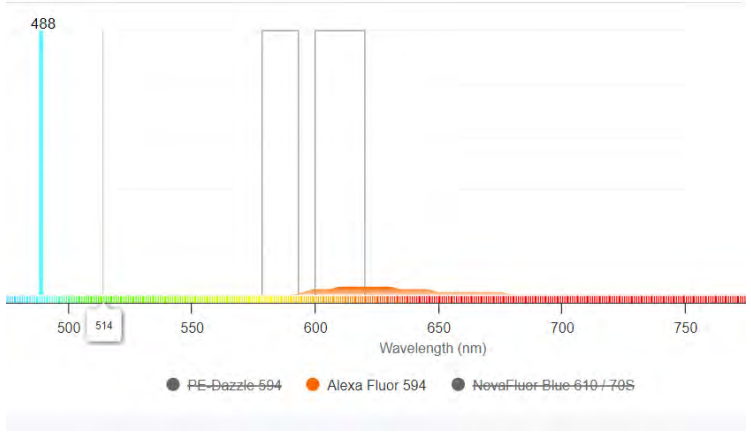
PE-dazzle594

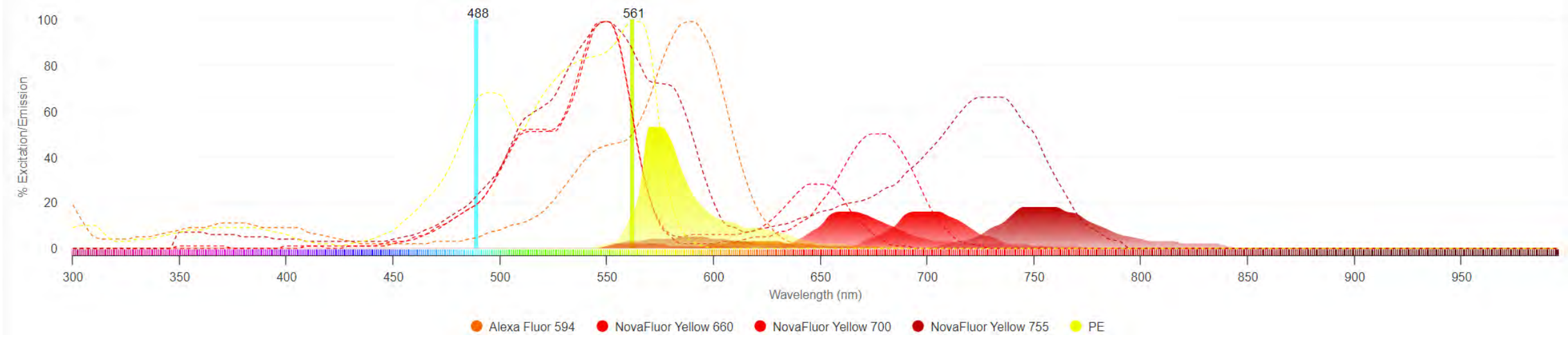


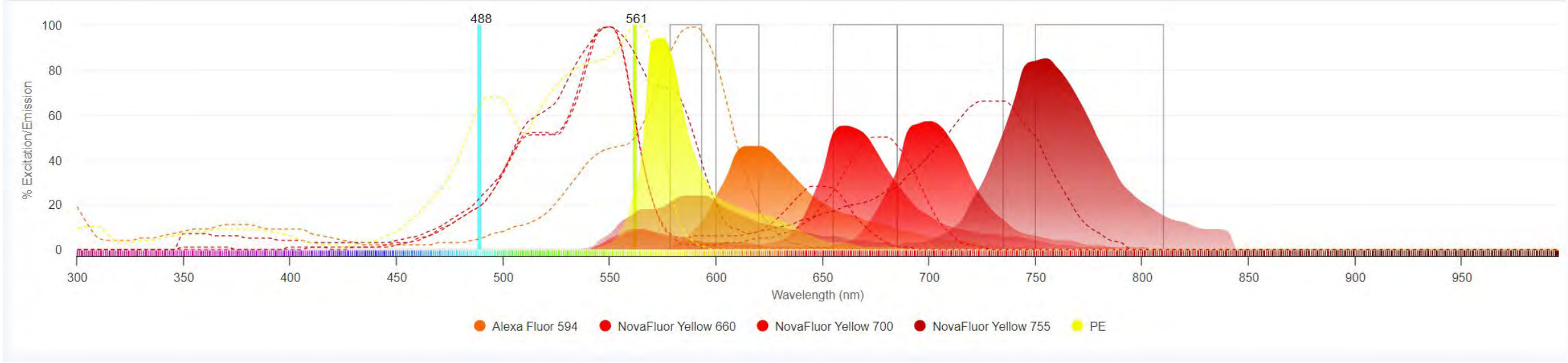
NFB-610-70S



AF594




488
561
100
80
60
40
20
0
% Excitation/Emission
300
350
400
450
500
550
600
650
700
750
800
850
900
950
Wavelength (nm)
Alexa Fluor 594
NovaFluor Yellow 660
NovaFluor Yellow 700
NovaFluor Yellow 755
PE


488
561
100
80
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40
20
0
% Excitation/Emission
300
350
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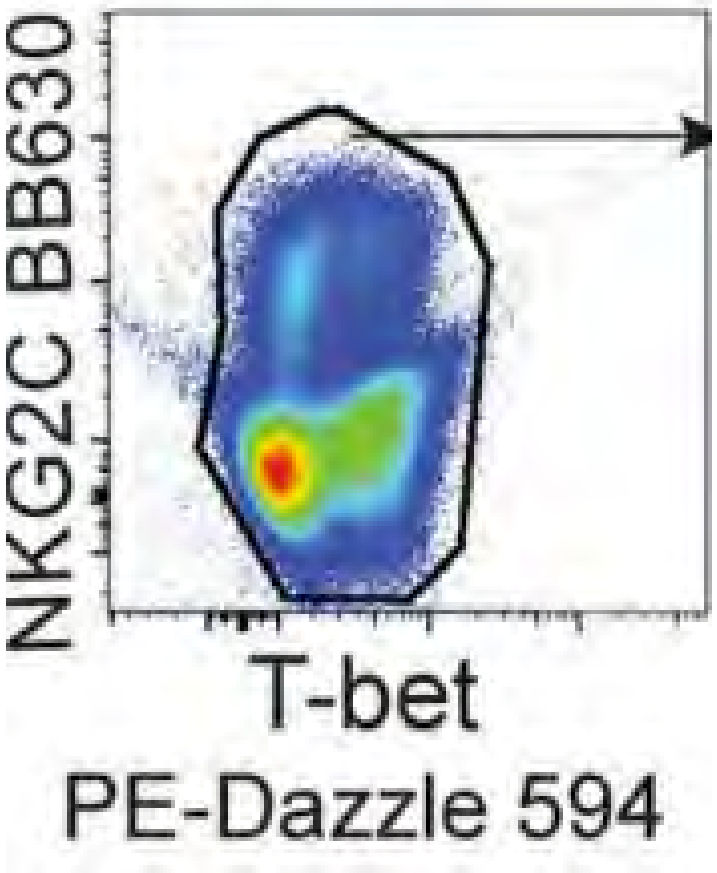
450
500
550
600
650
700
750
800
850
900
950
Wavelength (nm)
Alexa Fluor 594
NovaFluor Yellow 660
NovaFluor Yellow 700
NovaFluor Yellow 755
PE

Example II: what not to do…



https://doi.org/10.3389/fimmu.2019.02692

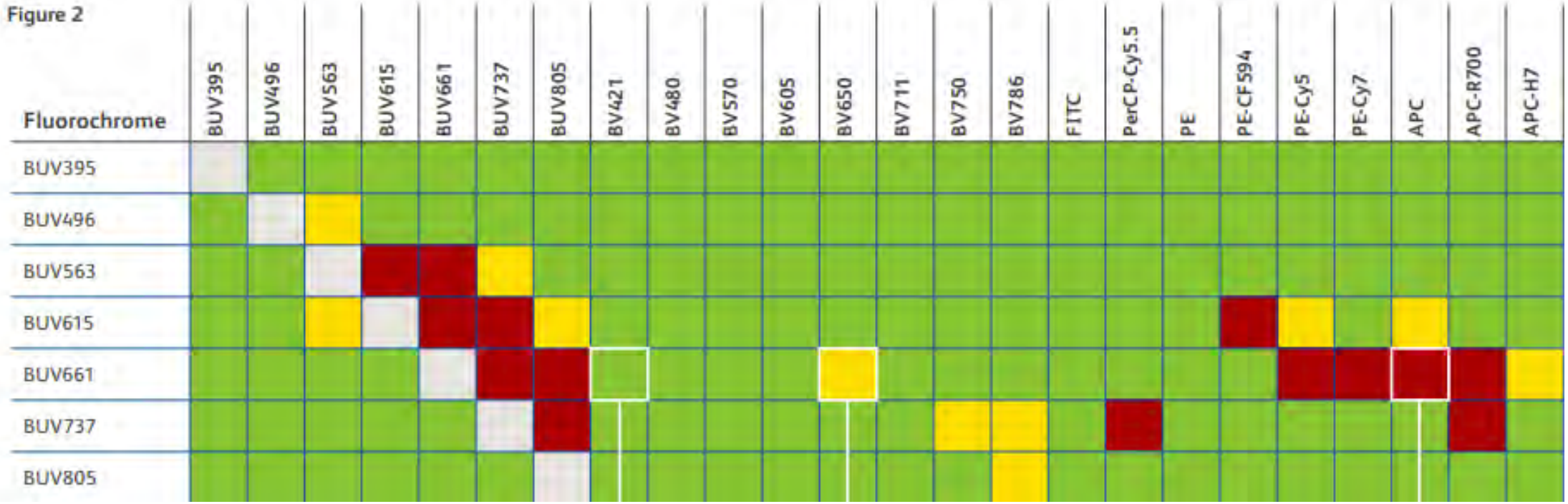
30 Color Ideal Fluorophore Choices:

UV Laser (355nm)	V Laser (405nm)	B Laser (488nm)	YG Laser (561nm)	R Laser (637nm)
SparkUV387 (BUV395)	BV421	BB515	PE	AF647* (APC)
FVS440 (DAPI)	BV480	NFB610-70S (BB630)	AF594 (PE-CF594)	APC-R700/AF700*/**
BUV496	BV570***	NFB660-40S (BB660)	NY660 (PE-Cy5)***	APC-eFluor780 (APC-H7)*****
BUV563	BV605***	BB700	NY700 (PE-Cy5.5)**	
BUV615	BV650*	RB744/BB755-P****	NY755***** (PE-Cy7)	
BUV661*	BV711	RB780/BB790-P****		
BUV737**	BV750			
BUV805	BV786*****			

Fluorophores with matching stars require more careful panel design choices when used in combination
NFB= Novafluor Blue
NY=Novafluor Yellow
FVS440=fixable viability stain

Parentheses indicate the channel name on the A5

Figure 2

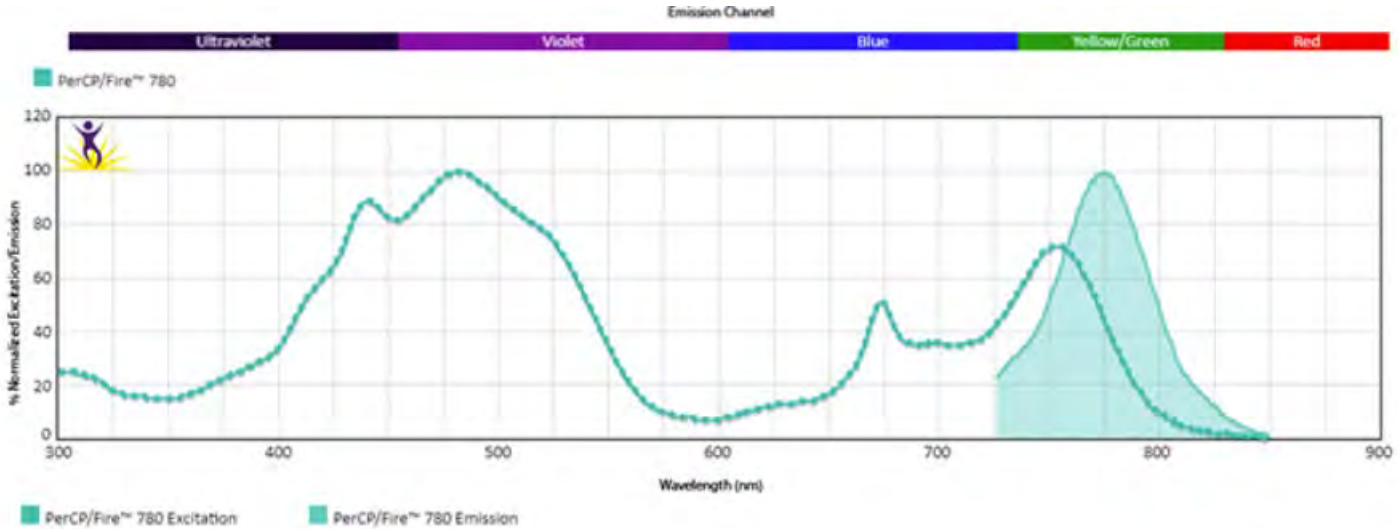

Fluorochrome
BUV395
BUV496
BUV563
BUV615
BUV661
BUV737
BUV805
BV421
BV480
BV570
BV605
BV650
BV711
BV750
BV786
FITC
PerCP-Cy5.5
PE
PE-CF594
PE-Cy5
PE-Cy7
APC
APC-R700
APC-H7

Questions?

Back to the Future- Unleashing your cytometer's spectral potential

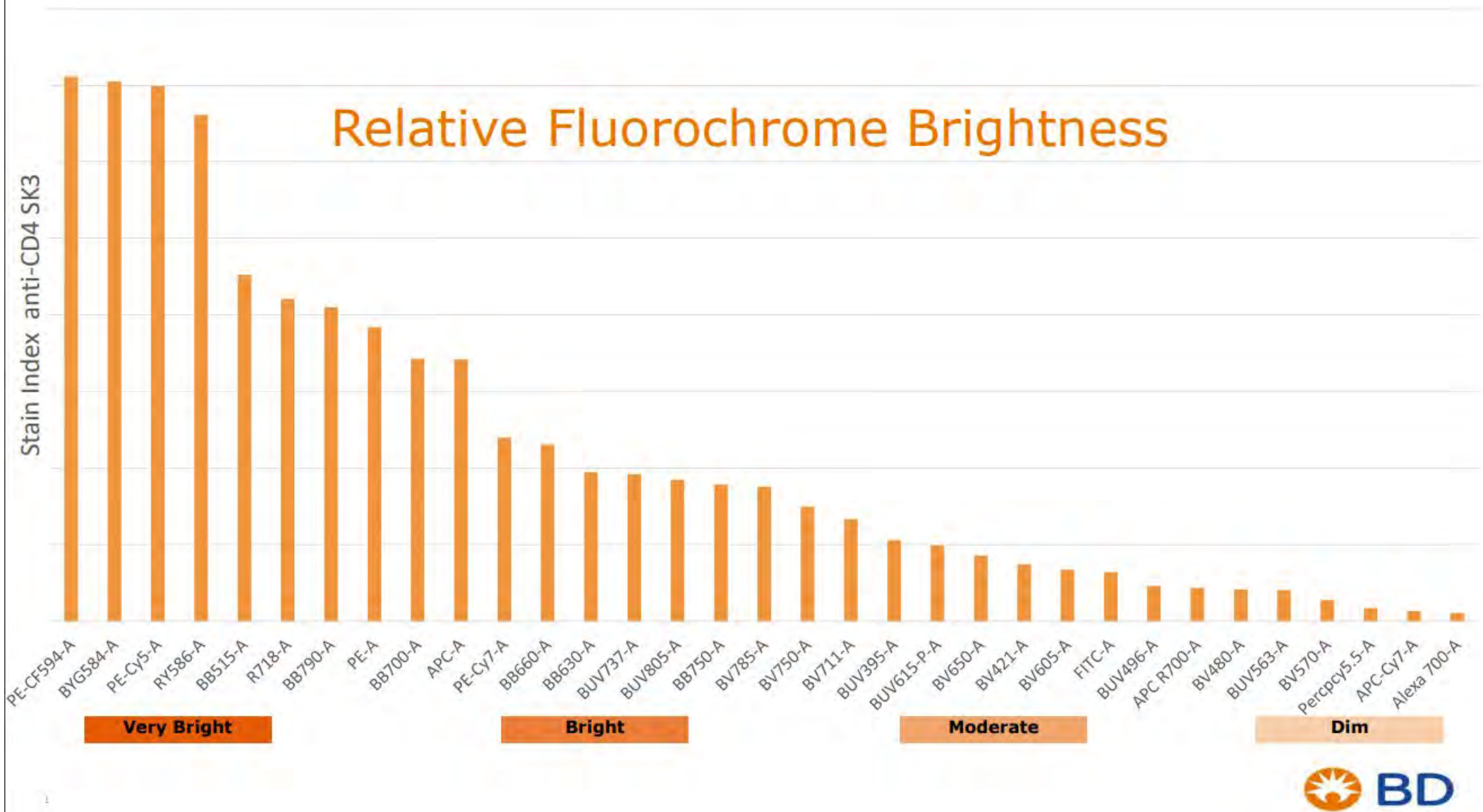
Christopher Hall[1], Hanan Ibrahim[1], Sam Thompson[1], Philip S Hobson[2], Jo-Anne Crofts[3], Peter Nobes [3], Steven Lim[2], Tony Burpee[3], Rachael V Walker[1]
1= Flow Cytometry Facility, Babraham Institute, Cambridge, CB22 3AT
2= Flow Cytometry Science Technology Platform, The Francis Crick Institute, 1 Midland Road, London, NW1 1AT
3= Applied Cytometry, Matrix Business Centre, Nobel Way, Dinnington, Sheffield S25 3QB.

Abstract
With the recent growth in spectral flow cytometry many laboratories are investing in new spectral flow cytometers in order to maximise the information gathered about every cell. This study hypothesised that traditional cytometers already within many laboratories may be used as spectral cytometers and have shown using a range of different cytometers that data acquired may be unmixed after acquisition.



Fluorophore at a Glance:

- Bright tandem dye ideal for antigens with low to moderate expression levels.
- Can be sensitive to high temperatures and alcohol-based fixatives like ethanol.
- Can be used with PE/Cyanine7 on instruments with blue and yellow/green lasers.


Relative Fluorochrome Brightness
Stain Index anti-CD4 SK3
PE-CF594-A
BYG584-A
PE-Cy5-A
RY586-A
BB515-A
R718-A
BB790-A
PE-A
BB700-A
APC-A
PE-Cy7-A
BB660-A
BB630-A
BUV737-A
BUV805-A
BB750-A
BV785-A
BV750-A
BV711-A
BUV395-A
BUV615-P-A
BV650-A
BV421-A
BV605-A
FITC-A
BUV496-A
APC R700-A
BV480-A
BUV563-A
BV570-A
Percpcy5.5-A
APC-Cy7-A
Alexa 700-A
Very Bright
Bright
Moderate
Dim