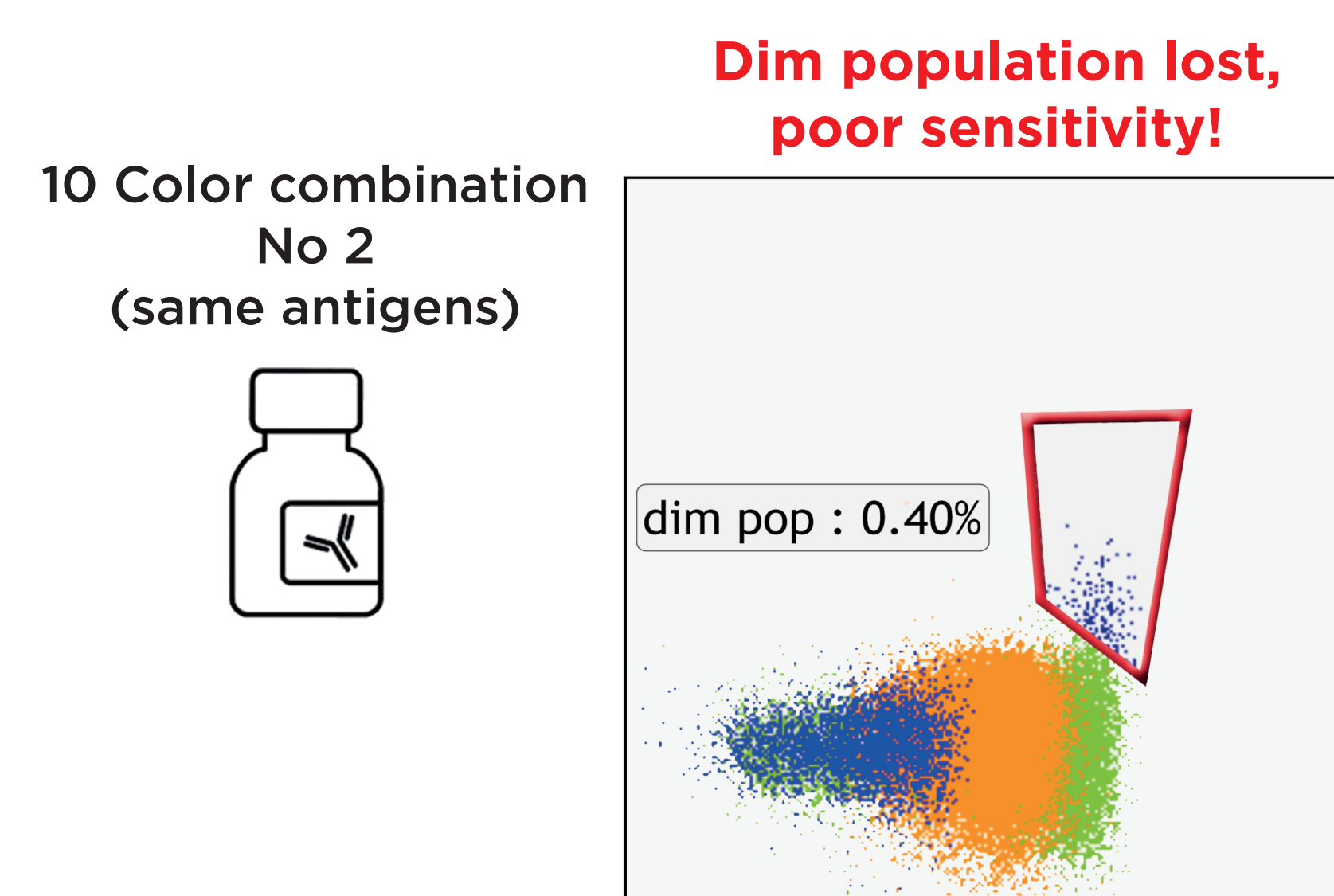
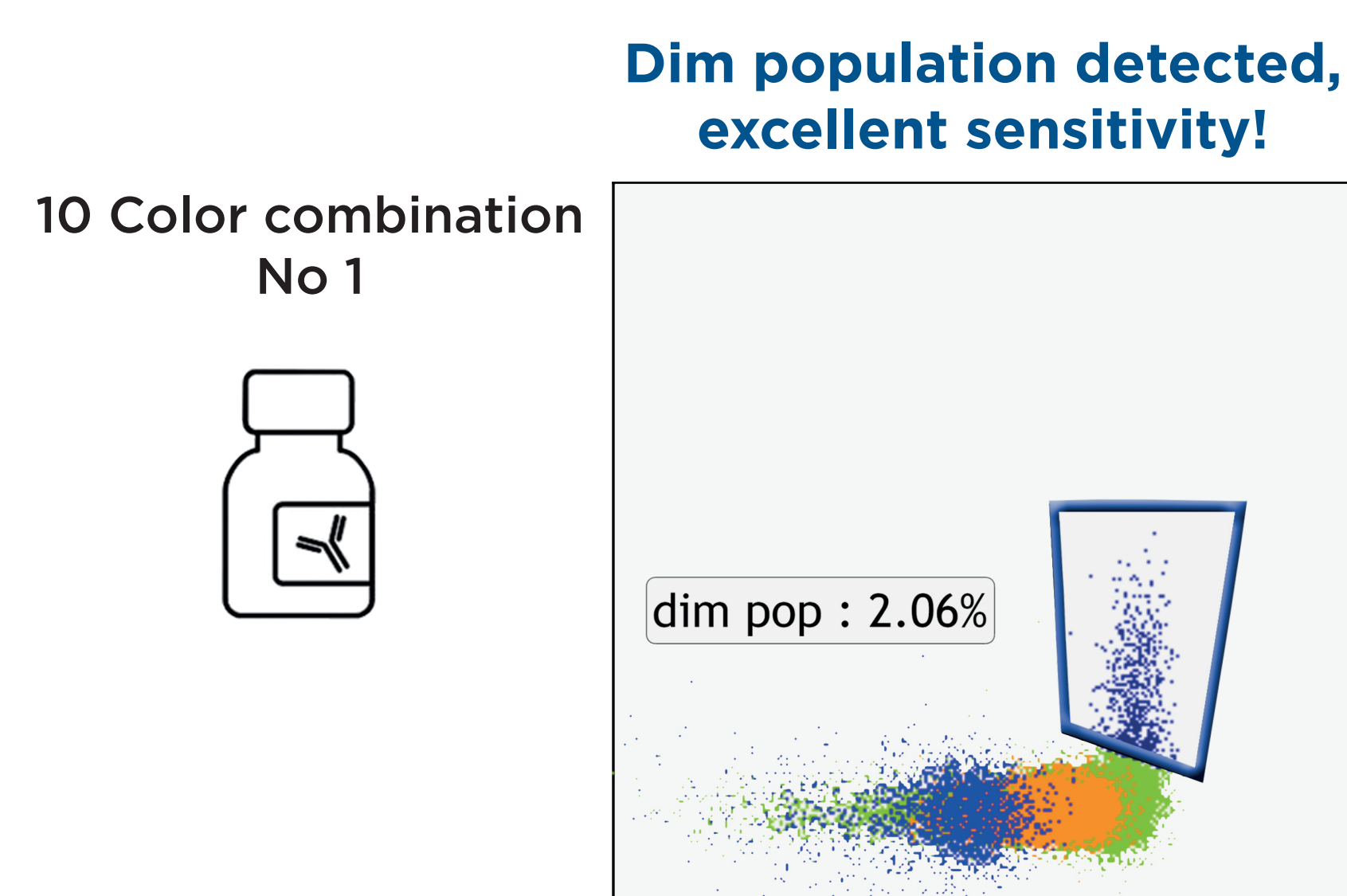


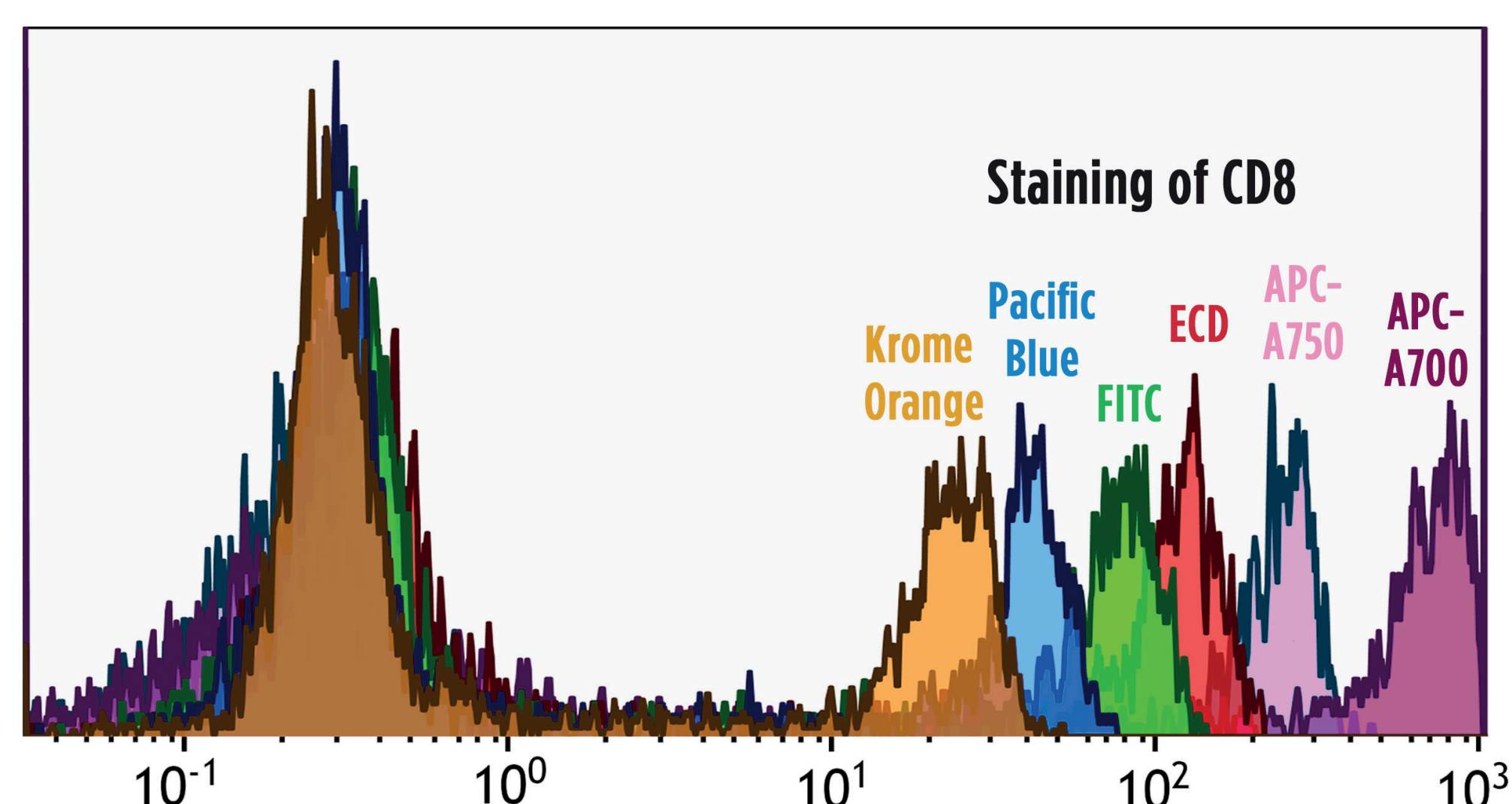
# How do I preserve sensitivity?

## ANTIBODY COMBINATIONS DETERMINE SENSITIVITY



STEP  
1

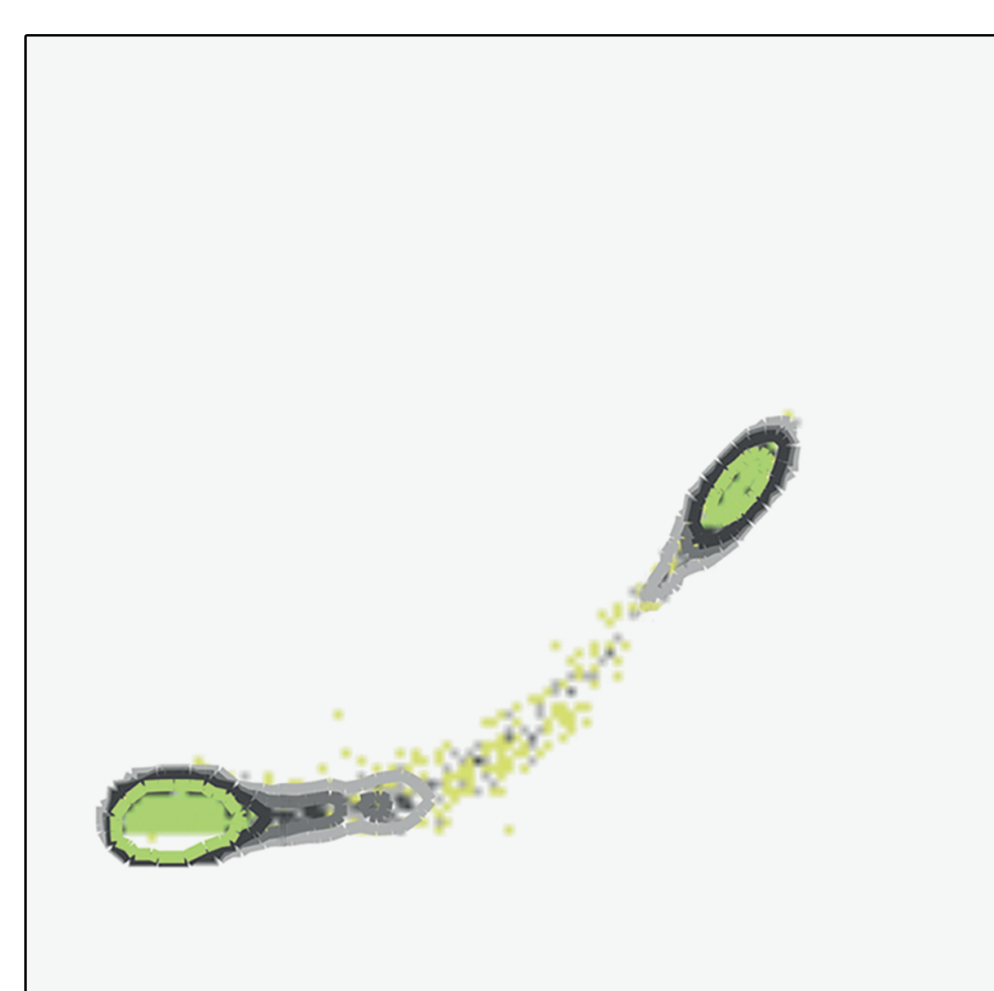
Use bright fluorochromes for weak antigens



Pacific Blue < FITC < ECD < A488 < PE < PC5 < PC5.5  
Krome Orange < A700 < APC-A750 < PC7 < APC < A647 < APC-A700

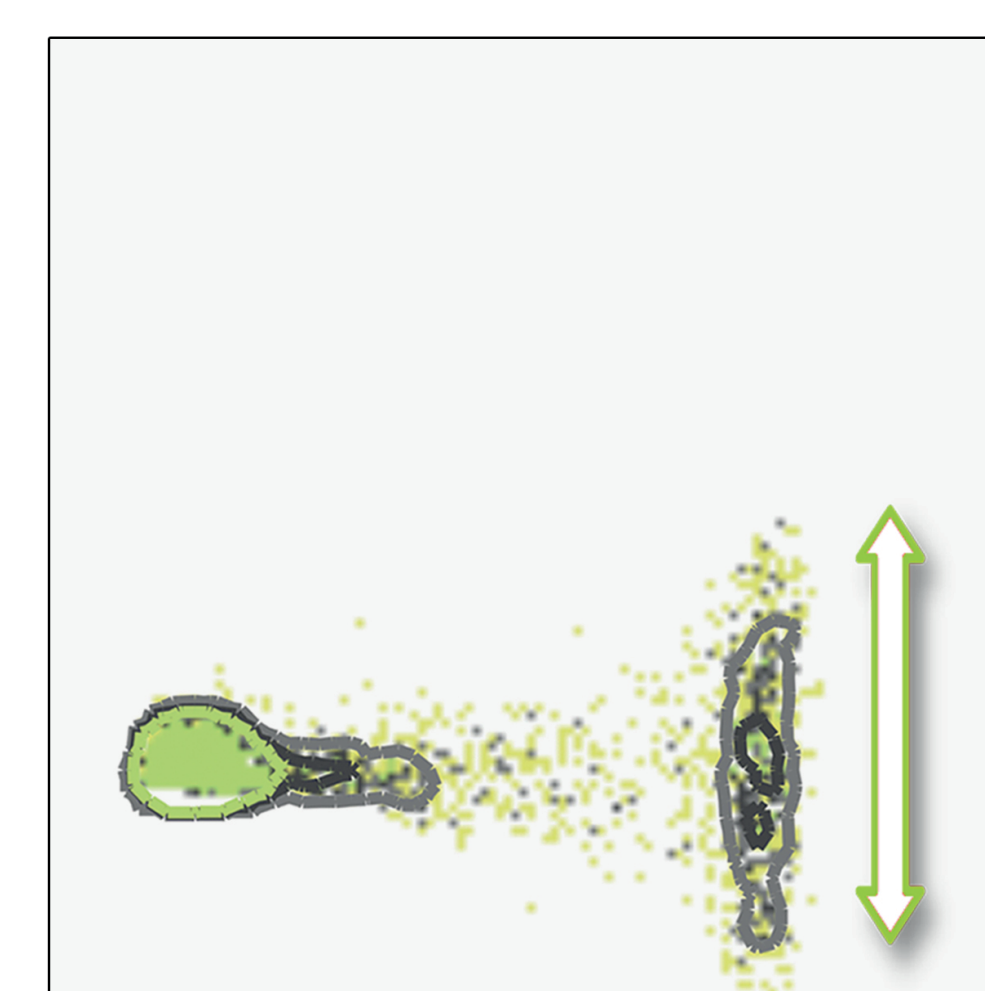
STEP  
2

Use dim fluorochromes for strong antigens



Compensation

Brighter emission means  
more spillover and  
more data spreading



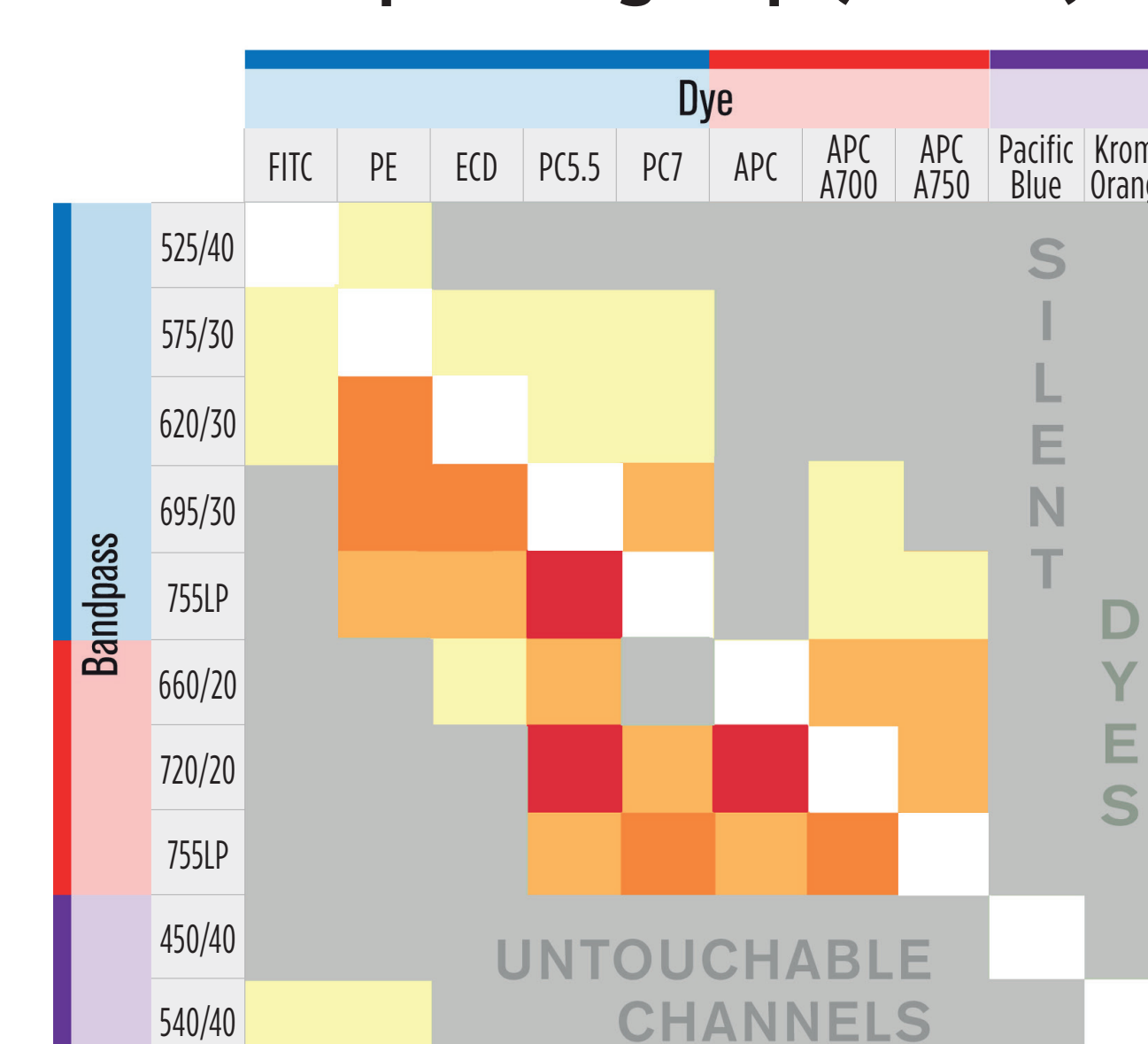
STEP  
3

Put weak antigens in untouchables channels  
Use bright fluorochromes for weak antigens

Increase of data spreading

Untouchable channels: no spillover/spreading from other dyes  
Silent dyes: no spillover/spreading into other channels

Spreading Map (Gallios)



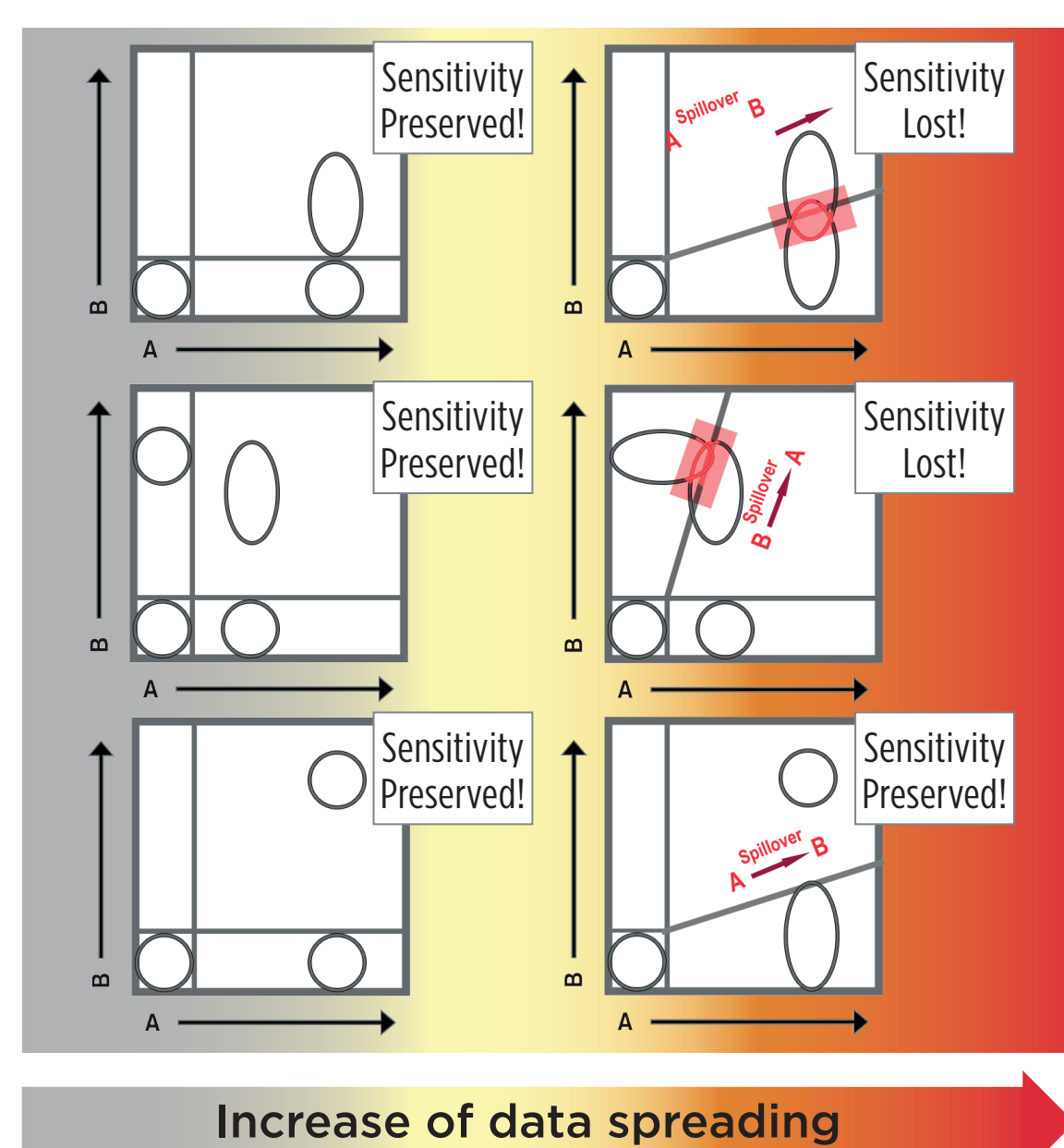
STEP  
4

Match co-expression pattern with spreading map above

Dim/modulated  
Co-expression  
B+/- on A++ cells  
or A+/- on B++ cells

Bright  
Co-expression  
B++ on A+ cells  
and on A- cells

Bimodal  
Co-expression  
B++ on A++



Parent Descendant

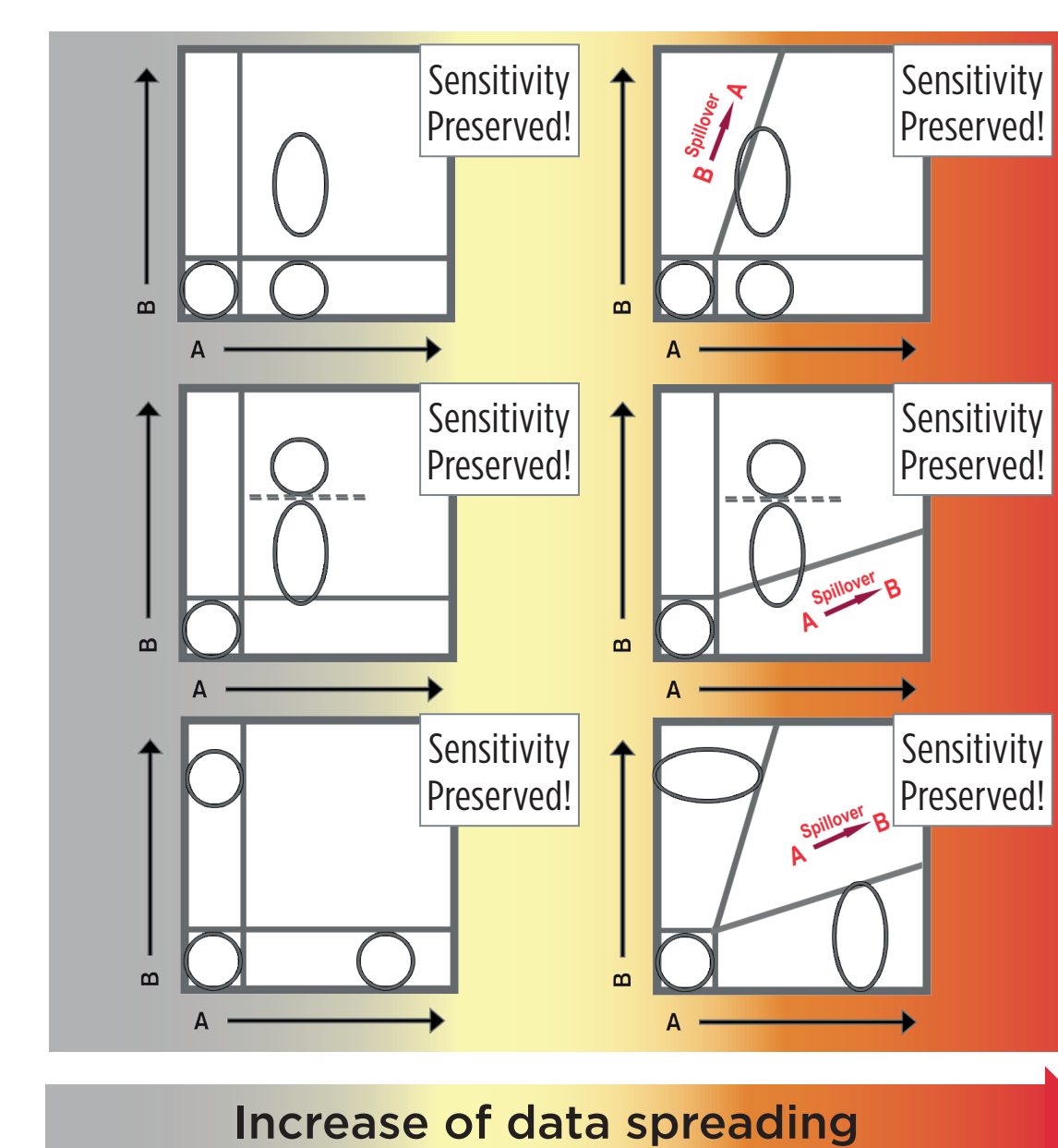
B+/++ on A+ cells  
but not on A- cells

Positive Threshold

Discriminate B++ vs B+  
on A+/++ cells,  
no discrimination B+ vs B-

Exclusion

A+/++ excludes B+/++



B  
R  
I  
G  
H  
T  
N  
E  
S

S  
P  
I  
L  
L  
O  
V  
E  
R

S  
P  
R  
E  
A  
D  
I  
N  
G

C  
O  
-  
E  
X  
P  
R  
E  
S  
S  
I  
O  
N

