Creating A Resolution Impact Matrix: A Tool for High Content Flow Cytometry Panel Design

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Background

With the advent of Flow Cytometry: Critical Assessment of Population Identification Method (FlowCAP), a lot of methods are coming to fruition on how to analyze high content flow cytometry data. Working from the other end of the experiment, there are several commercially available web-based and software programs to assist with panel design. However with different instrument optical configurations there can be differences between how well instruments resolve certain multicolor panels.

Here we use a method to generate an instrument-specific, and fluorochrome specific tool to assist with high content flow cytometry panel design: the Resolution Impact Matrix.

Introduction

In multicolor flow cytometry, after compensation is applied a measurement error from fluorescence spill over becomes apparent by spreading of a negative population. This spillover spread is instrument and reagent specific and will impact sensitivity in a given parameter[1].

To identify resolution of a given parameter, and the degree to which a given fluorochrome will impact sensitivity in a double-positive population, we demonstrate the generation of a resolution impact matrix for the Fortessa X50: a 5-laser, 30 parameter instrument at University of Nebraska Medical Center.

Method

To develop the RIM we first had to identity every parameter's stain index: the degree to which a single parameter positive can be resolved from a negative. To do this we obtained elutriated peripheral blood lymphocytes and separated into 29 tubes; 28 for single stains and 1 unstained tube. We stained samples individually with 28 unique fluorochromes each conjugated to an anti-CD4 antibody.

Running each sample on the Fortessa X50 we optimized voltages to maximize signal and minimize population spread, then recorded and compensated the data. We used the stain index as a measure of the degree to which a single parameter positive can be resolved from a negative. This was done by taking the positive median fluorescence intensity (MFI) of a single parameter, subtracting the negative population MFI, and dividing by the robust Standard Deviation (rSD) of the negative[2] (Figure 1 A, B). This parameter was named the resolution parameter, displayed along the X-axis.

We then took the remaining 27 samples and displayed them each on the Y-axis against the resolving parameter. Each Y-axis parameter was labelled an impacting parameter for a given resolution parameter. We used the single positive MFI identified, then took the negative MFI and rSD for each impacting parameter, to identify the new resolution for a double-positive population (Figure 1C, D).

A matrix was then generated, where every parameter was displayed against every other parameter. The resolution of each single parameter is displayed at the intersect of itself, and the resulting loss of resolution is displayed using a simple density color scale.

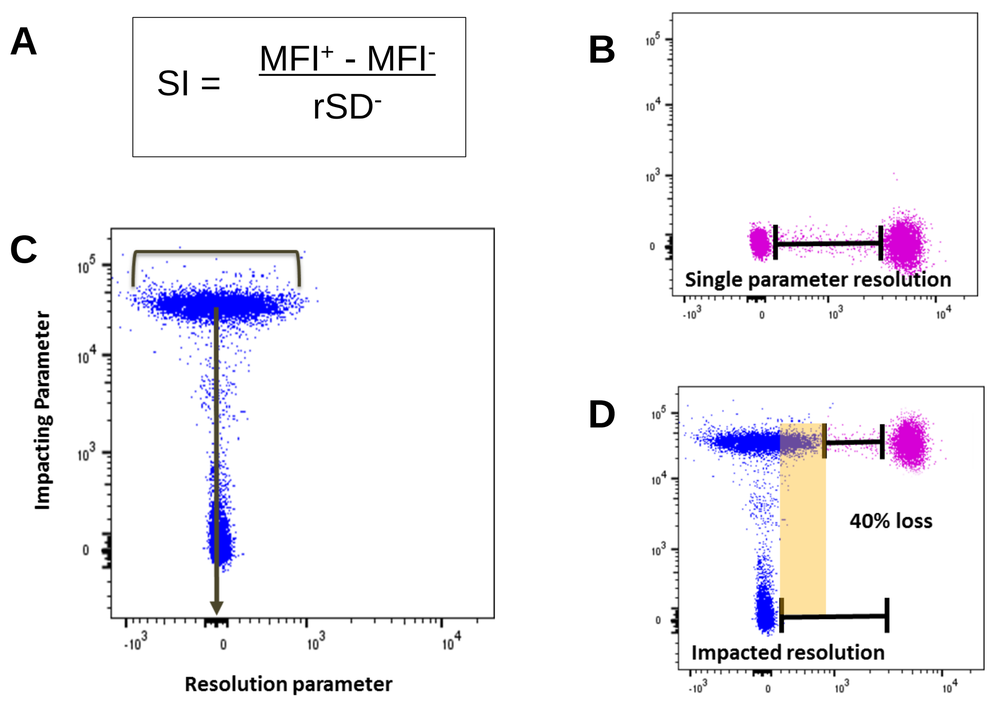

Figure 1: Using the stain index (a) to identify the resolution of a single parameter (b); calculating the resolution of an impacting parameter (c); and demonstrating the resulting impact of loss of resolution due to population spread (d).

Results

Primary Resolution Fluorochrome

RIM		UV-G	UV-F	UV-E	UV-D	UV-C	UV-B	UV-A	V-H	V-G	V-F	V-E	V-D	V-C	V-B	V-A	B-E	B-D	B-C	B-B	B-A	R-C	R-B	R-A	YG-E	YG-D	YG-C	YG-B	YG-A
		BUV395	BUV496	BUV563	BUV615	BUV661	BUV737	BUV805	BV421	BV510	BV570	BV605	BV650	BV711	BV750	BV785	FITC	PE	BB630	BB700	BB790	APC	AF700	APCCy7	BYG568	PECF594	PECy5	PECy5.5	PECy7
UV-G	BUV395	74																											
UV-F	BUV496		27																										
UV-E	BUV563			33																									
UV-D	BUV615				90																								
UV-C	BUV661					142																							
UV-B	BUV737						113																						
UV-A	BUV805							48																					
V-H	BV421								219																				
V-G	BV510									57																			
V-F	BV570										23																		
V-E	BV605											27																	
V-D	BV650												52																
V-C	BV711													57															
V-B	BV750														93														
V-A	BV785															47													
B-E	FITC																16												
B-D	PE																	154											
B-C	BB630																		368										
B-B	BB700																			231									
B-A	BB790																				179								
R-C	APC																					64							
R-B	AF700																						19						
R-A	APCCy7																							17					
YG-E	BYG568																								218				
YG-D	PECF594																									87			
YG-C	PECy5																										149		
YG-B	PECy5.5																											523	
YG-A	PECy7																												173

Secondary Impacting Fluorochrome

RESOLUTION IMPACT SCALE: 0 10 20 30 40 50 60 70 80 90 100

Figure 2: The Resolution Impact Matrix (RIM), with primary resolution fluorochrome displayed in the top row. The RIM shows how well a parameter resolves a positive from background at it's own intersect (black squares), and the impact each parameter has on resolution of a parameter when looking at a double-positive population. The impact on resolution is identified by a colored square along the row; the more red colors identifying parameters where resolution is highly impacted and green identifying parameters with little or no impact. The resolution impact scale is identified as percent loss from a single-parameter resolution.

Conclusion

The Resolution Impact Matrix is straight forward to generate, and is an instrument and fluorochrome specific tool useful for assisting with high content flow cytometry panel design. The two features from the RIM are:

1) Identifying high and low resolving parameters
2) Identifying parameters where there is high and low impact on resolution in a double-positive population

These are useful for identifying high resolving parameters for dim expressing markers, and pairing these with co-expressing cellular markers on low impacting resolution markers. Also, areas of high impact can be leveraged by pairing exclusive cellular markers in areas of high resolution impact where the impact is inconsequential.

References and Acknowledgements

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