Jump-start your experimental design with published antibody and reagent panels

Optimized multicolor immunofluorescence panels (OMIPs).

Coined by Roederer and Tarnok [1], an optimized multicolor immunofluorescence panel (OMIP) refers to a thoroughly tested and validated set of antibodies and reagents that can be used together for the multicolor characterization or evaluation of a specific cell state or response. For example, OMIP-001 is optimized for evaluating the quality and phenotype of Ag-responsive human T cells [2]; OMIP-009 is optimized for characterizing the immunological response of human T cells [3].

Published in the journal *Cytometry Part A* (Wiley Online Library), the first group of OMIPs are designed for flow cytometry, but an OMIP can potentially be defined for image cytometry, fluorescence microscopy, and other polychromatic fluorescence-based methods. The development and publication of these OMIPs not only helps to alleviate the burden of panel development and optimization by providing the technical details and experimental conditions used to optimize each panel (details usually omitted in published research reports), but also creates an online repository for OMIPs so that all researchers can easily search and access the information. Moreover, their publication provides a process for peer review of optimized panel data, as well as a platform where researchers can get recognition and credit for the amount of work and effort it takes to develop an OMIP [4].

Components of an OMIP

By definition, an OMIP publication includes all of the necessary information required for the execution of the panel of interest. The publication contains an overview of the purpose of the panel, a listing of antibody clones and fluorophore combinations, information on sample type, and similarities to any existing OMIPs; a representative figure shows experimental results, including the gating scheme. The supplemental

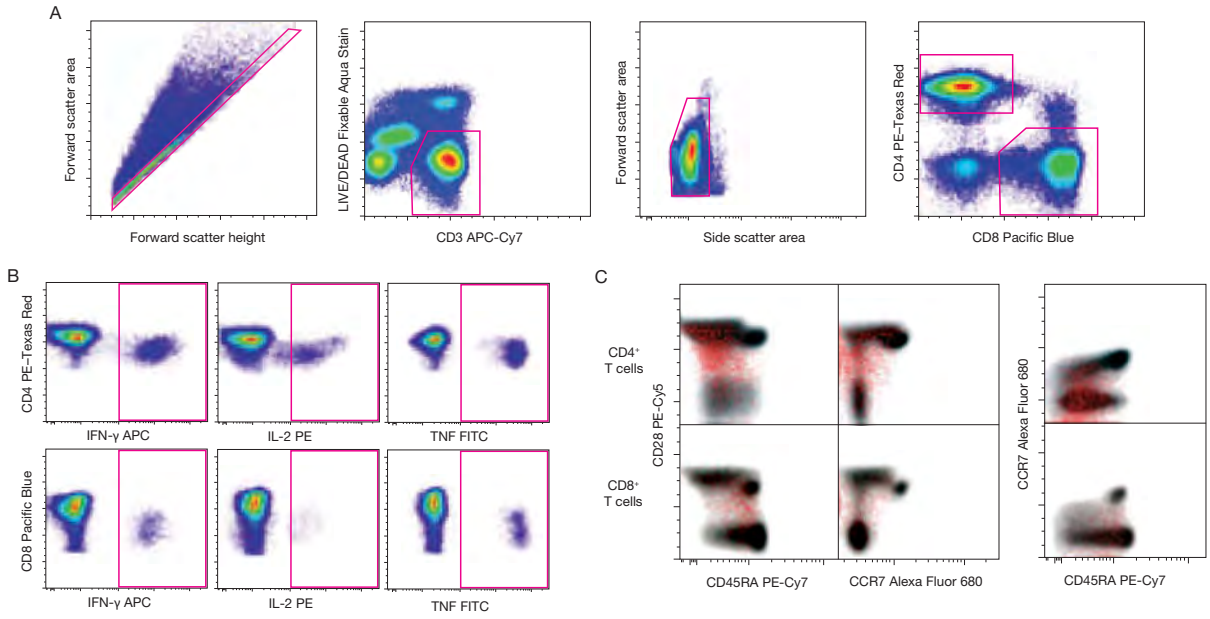

Figure 1. Example of immunophenotyping using OMIP-009. Data shown are from CMV1 donor cells stimulated with pp65 peptide pool (15-mers overlapping by 11). **(A)** Singlets are identified through the use of a forward scatter area (FSC-A) vs. forward scatter height (FSC-H) plot. Nonviable and $CD3^-$ cells are excluded, allowing for the selection of the live $CD3^+$ T cells only. A FSC-A vs. side scatter area (SSC-A) plot permits the additional removal of very low-scatter cells. The selected $CD3^+$ T cell population is then further delineated into $CD4^+$ and $CD8^+$ T cells. **(B)** Gated on either $CD4^+$ or $CD8^+$ T cells, the percentage of responding cells for each cytokine is determined. **(C)** Using Boolean gating logic in the FlowJo analysis program, the "or" function is used to create a single gate of all cytokine-producing cells from a combination of existing cytokine gates, i.e., IFN-γ^+ or IL-2^+ or TNF^+. Thus any cell that makes one or more cytokines is included in the gate. The total cytokine response (red) is then overlaid onto its respective $CD4^+$ (top) or $CD8^+$ (bottom) T cell lineage (gray) to identify the maturation and activation phenotype of the responding cells. Reprinted by permission from John Wiley & Sons Inc: Lamoreaux L, Koup RA, Roederer M (2012) OMIP-009: Characterization of antigen-specific human T-cells. *Cytometry A* 81:362–363.

material provides the developmental strategy, a detailed staining protocol, and technical information on antibody conjugate titration and panel optimization. The nomenclature used to identify each OMIP began with "OMIP-001" [2], and the numerical designation continues to increase as new OMIPs are peer-reviewed and then published in *Cytometry Part A*. To date, the majority of the OMIPs published have been developed for flow cytometry, which is likely due to the complexity of immunophenotyping studies performed using the platform.

An example of an OMIP that was developed for flow cytometry is OMIP-009, which was designed to study the human T cell immunological response to patient vaccination [3]. The list of labeling reagents used for the study and the corresponding products from Thermo Fisher Scientific can be found in Table 1. The data acquired using the optimized conditions for this panel are shown in Figure 1 [3].

Getting started with panel design

OMIP publications enable researchers to save a significant amount of time and money in the creation of their own panels. Even if the OMIP is not a perfect fit for a particular study, reviewing the strategy employed by other researchers in the development of a similar OMIP could prove invaluable to the design of the new panel. When developing a new panel for flow cytometry or other polychromatic fluorescence-based method, other considerations include:

- Biology of the system: Information about the cell populations, antigen density, and marker co-expression will help drive the gating strategy.
- Instrumentation: The optical configuration of the instrument, including excitation wavelengths and emission filters available, will dictate the detection strategy.
- Antibody characteristics: The specific antibody clones, chosen after titrating each antibody conjugate for optimal staining index in the panel, will help to maximize the resolution of different cell states and cell types.
- Fluorophore characteristics: Achieving the ideal reagent brightness will depend on the fluorophore's extinction coefficient and quantum yield and the instrument's excitation source intensity and fluorescence collection efficiency, as well as on the spillover spread matrix, compensation requirements, and autofluorescence in the system.

There are several resources available to help you get started with your own panel optimization [5–11], including a short article published in *BioProbes 71* called "Flow Cytometry Panel Design: The Basics" [8], which you can find at thermofisher.com/bp71. For a complete listing of published OMIPs, as well as information on how to publish an OMIP, go to thermofisher.com/omipbp74. ■

References

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11. Data files from many of the OMIPs are free and accessible online at http://flowrepository.org

Table 1. OMIP-009: Characterization of antigen-specific human T cells [3] and comparable products from Thermo Fisher Scientific.

Reagents used for OMIP-009 [3]			Comparable Thermo Fisher Scientific products				
Marker	Clone	Fluorophore	Marker	Clone	Fluorophore	Quantity	Cat. No.
IFN-γ	B27	APC	IFN-γ	B27	APC	500 µL	MHCIFG05
IL-2	MQ1-17H12	PE	IL-2	MQ1-17H12	PE	500 µL	RHCIL204
TNF	Mab11	FITC	TNF	Mab11	FITC	50 µg	A18469
CD3	SP342	APC-Cy®7	CD3	UCHT1	APC-Cy®7	100 µg	A15440
CD4	T4	ECD (PE–Texas-Red™)	CD4	S3.5	PE–Texas Red™	0.5 mL	MHCD0417
CD8	RPA-T8	Pacific Blue™	CD8	3B5	Pacific Blue™	500 µL	MHCD0828
CD45RA	L48	PE-Cy®7	CD45RA	HI100	PE-Cy®7	25 tests	A16358
CD28	CD28.2	PE-Cy®5	CD28				Not available
CCR7 (CD197)	150503	Alexa Fluor™ 680	CCR7 (CD197)				Not available
Dead cells	Not applicable	LIVE/DEAD™ Fixable Aqua Stain	Dead cells	Not applicable	LIVE/DEAD™ Fixable Aqua Stain	80 tests 200 tests 400 tests	L34965 L34957 L34966