



# OMIP 077: Definition of all principal human leukocyte populations using a broadly applicable 14-color panel

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## Abstract

This Optimized Multicolor Immunofluorescence Panel was designed to identify and quantify all principal leukocyte populations in human blood using a minimum number of markers. We achieved this goal using a carefully selected combination of 14 surface markers compatible with standard flow cytometric instruments and accessible to a particularly large research community. Optimized for use in whole blood, this panel allows polymorphonuclear cell identification, supports live cell recovery, and is well-suited for absolute cell counting applications in the original in vivo volume. Panel performance and the separation of populations are high, and virtually no cells remain undefined after gating. Besides the identification of neutrophils, eosinophils, basophils, T cells, natural killer cells, B cells, plasma cells, monocytes, myeloid dendritic cells and plasmacytoid dendritic cells, this panel also covers progenitor cells and may therefore be attractive for stem cell researchers. Envisioned applications of this panel include immune monitoring within clinical trials, initial discovery to inform subset-targeted panels, and clinical diagnostics. In summary, this panel offers a broadly applicable platform for immune cell identification, quantification and characterization in human samples, particularly whole blood.

## KEYWORDS

absolute cell counting, flow cytometry, immune monitoring, immune phenotyping, leukocyte, PBMC, whole blood

## 1 | PURPOSE AND APPROPRIATE SAMPLE TYPES

**Abbreviations:** APC, Allophycocyanin; BV, Brilliant Violet; FCM, flow cytometry; FITC, fluorescein isothiocyanate; FSC, forward scatter; MCM, mass cytometry; mDC, myeloid dendritic cell; Monocyte (c/nc), classical/nonclassical monocyte; MZB, marginal zone B; NK, natural killer; NKT, natural killer T; OMIP, optimized multicolor immunofluorescence panel; opt-SNE, optimized t-distributed stochastic neighbor embedding; PBMC, peripheral blood mononuclear cell; pDC, plasmacytoid dendritic cell; PE, phycoerythrin; PerCP, peridinin-chlorophyll-protein complex; SSC, side scatter; WB, whole blood.

Maximilian Boesch and Martina Sykora contributed equally to this work.

The present optimized multicolor immunofluorescence panel (OMIP) provides for the detection and in-depth analysis of all major leukocyte subsets in human whole blood (WB) using a single flow cytometry (FCM) panel with just 14 colors (Table 1). The panel offers an easy, fast and reproducible way of comprehensive immune cell profiling for various purposes including translational research and companion

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immune monitoring of clinical trials. Of note, the panel has been specifically designed to analyze erythrocyte-depleted WB, hence allowing the characterization of polymorphonuclear cells in addition to the widely used peripheral blood mononuclear cells (PBMCs). The use of WB instead of density gradient-enriched material further permits absolute cell counting in the original sample volume. Other notable assets are the detection of hematopoietic progenitors as well as the possibility for myeloid dendritic cell subset discrimination. In contrast to high-dimensional FCM or mass cytometry (MCM) panels, this 14-color panel does not require high-end instrumentation and is therefore accessible to a particularly broad research community.

This OMIP has been optimized in terms of (i) phenotyping depth (goal: to cover all major leukocyte subsets in WB with a minimum number of markers), (ii) the antibodies used (goal: to reduce unspecific binding), (iii) the fluorochromes used (goal: to maximize the staining

index while limiting channel spillover), (iv) the expression levels of the individual antigens (goal: to use bright fluorochromes for lowly expressed antigens and vice versa), and (v) user-friendliness and applicability (goal: to provide an easy and fast method compatible with standard FCM instruments).

## 2 | BACKGROUND

High-dimensional single cell analysis using FCM [1, 2] or MCM [3] has provided unprecedented insights into the cellular makeup of heterogeneous samples. However, considering that the number of theoretical populations increases exponentially with every marker dimension added [4, 5] and that the complexity of data analysis and interpretation rises alongside, the benefit of high-dimensional cytometry for a given study has to be carefully weighted. This is especially true for clinical applications where immune profiling of a high number of samples shall robustly assess the influence of a tested medicine on the abundance and phenotype of the major immune cell lineages even in a multicenter setting [6] rather than analyzing a maximum number of populations or deciphering developmental trajectories [7]. Hence, there is room for limited-color FCM panels nevertheless capable of resolving all major leukocyte subsets in humans. The present OMIP shall occupy this niche and leverage the low-threshold access to comprehensive FCM-based immune phenotyping in laboratories around the globe. Notably, the present OMIP was specifically designed to produce clear populations and be applicable to multiple instruments from various manufacturers even without normalization.

**TABLE 1** Summary table for application of this OMIP

Purpose	Comprehensive phenotyping of leukocyte subsets in WB using flow cytometry with just 14 colors
Species	Human ( <i>Homo sapiens</i> )
Cell types	Leukocytes from human WB (anticoagulated, erythrocyte-depleted)
Cross references	OMIP-023, OMIP-024, OMIP-042, OMIP-051, OMIP-063

Abbreviations: OMIP, optimized multicolor immunofluorescence panel; WB, whole blood.

**TABLE 2** Summary table of antibodies used in this OMIP

Specificity	Fluorochrome	Clone	Purpose/definition of
CD1c	PE	AD5-8E7	CD1c <sup>+</sup> mDCs, MZB cells
CD3	BV605	OKT3	T cells
CD14	BV711	MΦP9	Monocytes (c), intermediate monocytes, monocytes (nc)
CD15	PerCP-Cy5.5	HI98	Eosinophils
CD16	APC-eFluor780	ebioCB16	Neutrophils, intermediate monocytes, monocytes (nc)
CD19	APC-R700	HIB19	B cells, plasma cells
CD34	FITC	HPCA-2	Progenitor cells
CD38	BV421	HIT2	Plasma cells, basophils
CD45	BV480	HI30	Pan-leukocytes
CD56	PE-Cy7	NCAM16.2	NK cells
CD123	BV650	7G3	Basophils, pDCs
CD141	APC	AD5-14H12	CD141 <sup>+</sup> mDCs
CD193	PE-CF594	5E8	Eosinophils, basophils
HLA-DR	BV786	G46-6	Neutrophils, monocytes (c), intermediate monocytes, monocytes (nc), B cells, plasma cells, CD1c <sup>+</sup> mDCs, CD141 <sup>+</sup> mDCs

Abbreviations: mDC, myeloid dendritic cell; monocyte (c/nc), classical/non-classical monocyte; MZB, marginal zone B cell; NK, natural killer; OMIP, optimized multicolor immunofluorescence panel; pDC, plasmacytoid dendritic cell.

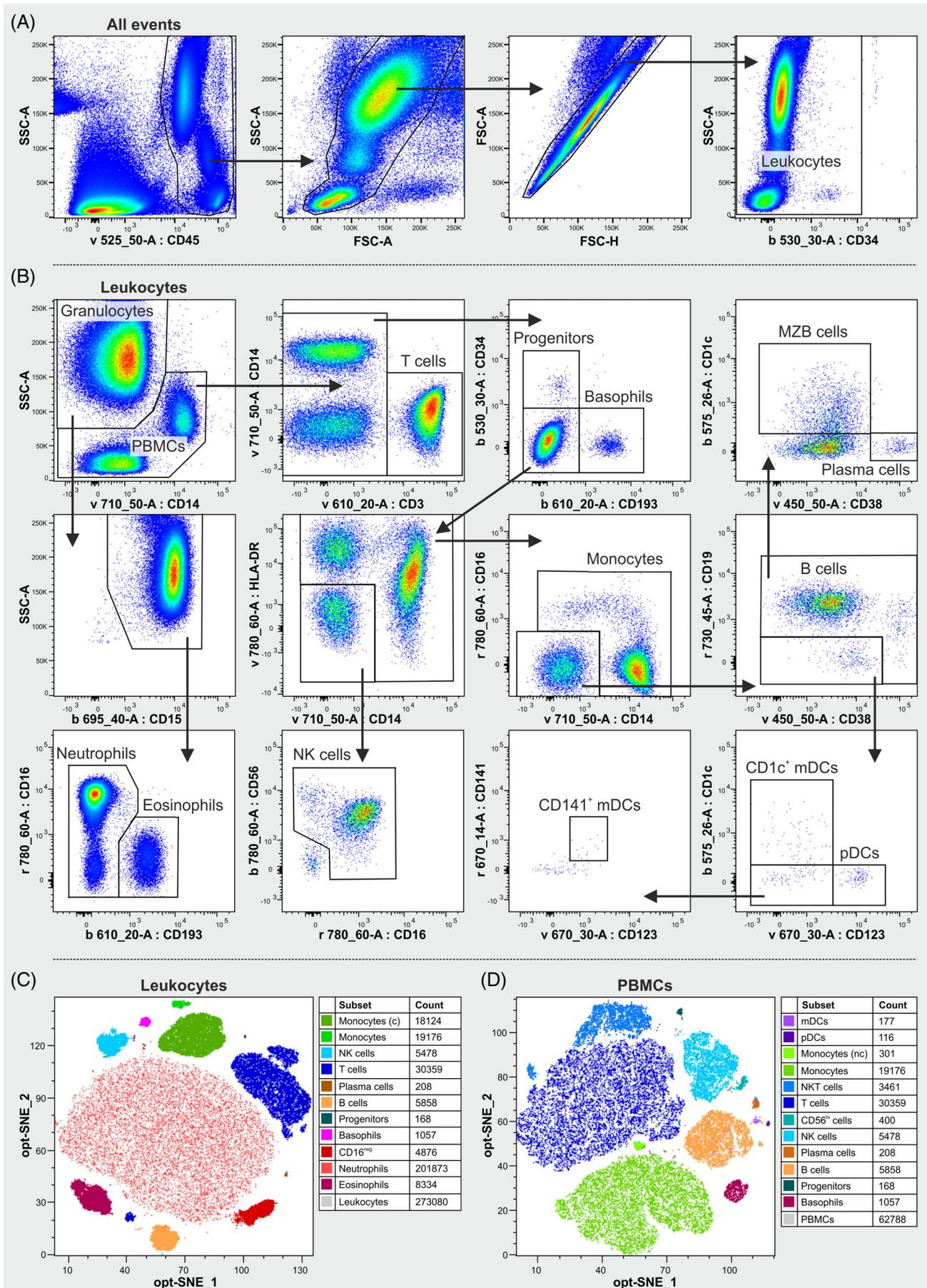


FIGURE 1 Legend on next page.

After several months of development and optimization (see Online supplement), we arrived at the 14-color panel shown in Table 2, exclusively targeting surface markers. After general gating steps involving (i) the prospective identification of pan-leukocytes using CD45 expression, (ii) the exclusion of dead cells and debris based on FSC/SSC characteristics, (iii) the exclusion of doublets and aggregates using the different signals of FSC and, if applicable, (iv) the exclusion of counting bead contamination (Figure 1(A)), PBMCs are separated from granulocytes using a bivariate SSC/CD14 plot (Figure 1(B)). CD15<sup>+</sup> granulocytes are subsequently divided into CD193<sup>-</sup>/CD16<sup>+/-</sup> neutrophils and CD193<sup>+</sup> eosinophils. Within the CD3<sup>+</sup> T cell-excluded PBMC compartment, CD34<sup>+</sup> progenitor cells as well as CD193<sup>+</sup> basophils are defined. The bulk of CD34/CD193 double-negative cells contain HLA-DR<sup>-</sup>/CD14<sup>-</sup> natural killer (NK) cells expressing CD56 (and for the most part CD16) as well as populations of HLA-DR/CD14 single- or double-positive cells. This latter fraction comprises monocyte populations that express CD14 and/or CD16 as well as a population of CD14/CD16 double-negative cells. CD14<sup>-</sup>/CD16<sup>-</sup> cells mostly consist of CD19/CD38 single- or double-positive cells in turn representing CD19<sup>+</sup> B cells, CD38<sup>+</sup> plasma cells and CD1c<sup>+</sup> marginal zone B (MZB) cells. The CD19<sup>-</sup>/CD38<sup>dim</sup> fraction contains both CD1c<sup>+</sup> myeloid dendritic cells (mDCs) and CD123<sup>+</sup> plasmacytoid dendritic cells (pDCs). Finally, the CD1c/CD123 double-negative cell fraction contains CD141<sup>+</sup> mDCs. The discriminatory power of the panel is excellent as evidenced by optimized t-distributed stochastic neighbor embedding (opt-SNE) analysis which shows unambiguous separation of the various cell populations downstream of the leukocyte (Figure 1(C)) or PBMC (Figure 1(D)) denominators. Moreover, the panel has a very high coverage such that virtually no cells remain undefined after gating. For the clear demarcation of individual populations, it is therefore essential that all populations be gated so as to exploit the panel's full potential and definitely avoid the false allocation of cells (particularly relevant for minor populations). Collectively, this carefully selected, limited-color flow panel allows for the unambiguous definition of all principal leukocyte subsets in human WB using a convenient and fast one-step/single-tube staining procedure. The single-tube nature of our assay not only is convenient and efficient, it also allows the analysis of very small amounts of blood (relevant for newborns) as well as the collection of as much information as possible. A comprehensive list of relative antigen expression for each marker for each cell type is provided in Online Table 4.

As per intent, the here presented panel does not target minor cell subsets downstream of respective denominator populations, such as regulatory T cells (downstream of CD3 [8]) and certain subsets of NK

cells and innate lymphoid cells (downstream of CD56 [9–11]). Instead, this panel is intended as a general and broadly applicable immune profiling platform that may also serve as a starting point for more specialized investigations based on subset-targeted panels, panels specific for cell activation/exhaustion, or panels discriminating effector from memory phenotypes. Nevertheless, this panel supports the collection of information beyond the main target populations it was designed for. As an example, the combination of CD3 and CD56 allows for the detection and quantification of NKT cells [12] and the combination of CD19 and CD1c enables MZB cell delineation [13] (Figure 1). In addition, the panel permits the analysis of HLA-DR expression on non-antigen-presenting cells such as T cells [14] and allows for the quantification of HLA-DR and CD38 double-positive cells, an activated T cell subpopulation, which has regained interest in the SARS-CoV-2 pandemic [15, 16].

We waived the use of a dead cell discrimination marker since cell viability in blood is generally high such that exclusion of the remaining dead cells by means of FSC/SSC characteristics was deemed appropriate and sufficient. However, this panel can be adopted in whole or in part to analyze leukocytes from other tissue sources and a dead cell discrimination marker may then be considered (especially after tissue disaggregation). The use of WB instead of buffy coats re-prioritizes polymorphonuclear cells and permits absolute cell counting in the original *in vivo* volume (Online Figure 5), thus reducing experimental variation especially in the case of minor cell populations [17]. Further assets are the detection of CD34<sup>+</sup> hematopoietic progenitor cells (highly relevant for leukemia stem cell research [18]) as well as the possibility for live cell recovery for functional testing.

In summary, we present a versatile, surface marker-only, 14-color flow panel for the prospective identification of all major immune cell lineages in human WB. Standard flow cytometric instruments of various brands support this panel, which is hence accessible to a particularly large number of researchers. Although a plethora of research questions can be addressed with this OMIP, envisaged key applications are (i) companion immune profiling of clinical trials and (ii) initial discovery paving the way for more specialized panels. Moreover, our panel may be adopted for use in clinical diagnostics based on novel 3-laser instruments such as 12-color FACSLyric (BD Biosciences) and 13-color DxFLEX (Beckman Coulter). In this case, select markers would need to be omitted and prime candidates for this purpose would be CD15 and CD193 considering that neutrophils and eosinophils can usually be sufficiently discriminated using SSC/autofluorescence/CD16 characteristics or, alternatively, be enumerated by a differential blood count.

**FIGURE 1** Gating strategy and performance of this OMIP. (A) General gating strategy for identifying single viable leukocytes in human WB. Note that the last gating step is only applicable when counting beads were included in the staining. (B) Exemplary gating strategy for identifying the principal cell populations downstream of the leukocyte denominator. (C) Opt-SNE plot showing the separation of leukocyte populations in human WB. (D) Opt-SNE plot showing the separation of PBMC populations in human WB. mDC, myeloid dendritic cell; MZB, marginal zone B; monocyte (c/nc), classical/nonclassical monocyte; NK, natural killer; NKT, natural killer T; OMIP, optimized multicolor immunofluorescence panel; PBMC, peripheral blood mononuclear cell; pDC, plasmacytoid dendritic cell; opt-SNE, optimized t-distributed stochastic neighbor embedding; WB, whole blood.

Note: Data as shown here were acquired on a 3-laser BD LSRFortessa flow cytometric instrument (BD biosciences) [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

### 3 | SIMILARITY TO OTHER OMIPs

This OMIP has a partially overlapping scope with OMIP-023 [19], OMIP-024 [20], OMIP-042 [21], OMIP-051 [1], OMIP-063 [22]. However, many of these OMIPs incorporate significantly more markers (OMIP-042, OMIP-051, OMIP-063) and are hence not compatible with standard FCM instruments. Furthermore, many of these OMIPs are optimized for PBMC analysis (OMIP-024, OMIP-042, OMIP-051, and OMIP-063) and therefore do not consider or prioritize polymorphonuclear cells. While the here presented OMIP does not emphasize cell subsetting downstream of principal denominator populations, it is certainly unique in detecting both CD34<sup>+</sup> progenitor cells and mDC divergence (CD1c<sup>+</sup> vs. CD141<sup>+</sup>).

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#### CONFLICT OF INTEREST

The authors have no potential conflicts of interest to declare. No medical writer or other non-author was involved in the preparation of the manuscript.

#### AUTHOR CONTRIBUTIONS

**Maximilian Boesch:** Conceptualization; data curation; formal analysis; validation; visualization; writing-original draft; writing-review & editing. **Martina Sykora:** Conceptualization; data curation; formal analysis; investigation; methodology; validation; visualization; writing-review & editing. **Silvia Gasteiger:** Investigation; writing-review & editing. **Florent Baty:** Formal analysis; resources; validation; writing-review & editing. **Martin H. Brutsche:** Formal analysis; resources; validation; writing-review & editing. **Sieghart Sopper:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; visualization; writing-original draft; writing-review & editing.

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#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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