

Design and Optimisation of a 39-colour Spectral Flow Cytometry Panel to Characterise Immune Response Dynamics in *Trypanosoma*, *Leishmania*, and *Plasmodium* Infections

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Understanding complex immune interactions during parasitic infections requires tools capable of capturing high-dimensional cellular data. Spectral flow cytometry provides a powerful platform to investigate these host-pathogen interactions. Unlike conventional flow cytometry, which relies on discrete optical filters, spectral cytometry captures the full emission spectrum of each fluorochrome, enabling the simultaneous use and accurate separation of 40-50 or more markers based on their unique spectral signatures. Such high-dimensional multicolour panels offer a comprehensive overview of immune cell composition, activation states, and immune mediators during infection. However, the development of these multiparameter panels requires careful fluorochrome selection to minimize spectral overlap and optimise signal resolution.

We developed and optimised a 39-colour spectral flow cytometry panel designed to widely profile both myeloid (eosinophils, neutrophils, dendritic cells, and macrophages) and lymphoid cells (natural killer cells, $\alpha\beta$ T cells, $\gamma\delta$ T cells, and B cells), including various activation markers, immune checkpoint molecules, and intracellular cytokines. Fluorochromes were selected based on antigen density, brightness, and spectral overlap, followed by systematic antibody panel optimisation and quality control on a Cytex® Aurora flow cytometer.

This panel was specifically designed for use in infection models of trypanosomiasis, leishmaniasis and malaria, caused by the major protozoan genera *Trypanosoma*, *Leishmania*, and *Plasmodium*, respectively. These pathogens elicit highly distinct yet overlapping immune responses, making them ideal for comparative immunological analyses. This 39-colour panel enables comprehensive immune profiling across these infection models, facilitating the identification of shared and pathogen-specific host immune pathways. Its robust and flexible design makes it a versatile platform for investigating a broad range of parasitic infections, with potential applicability to other disease models.