Standardized high-dimensional spectral cytometry protocol and panels for whole blood immune phenotyping in clinical and translational studies

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Flow cytometry is the method of choice for immunophenotyping in the context of clinical, translational, and systems immunology studies. Among the latter, the Milieu Intérieur (MI) project aims at defining the boundaries of a healthy immune response to identify determinants of immune response variation. New generation spectral cytometers now enable high dimensional immune cell characterization from small sample volumes. Therefore, for the MI 10-year follow-up study, we have developed two high-dimensional spectral flow cytometry panels for deep characterization of innate and adaptive whole blood immune cells (35 and 34 fluorescent markers, respectively). We have standardized the protocol for sample handling, staining, acquisition, and data analysis. This approach enables the reproducible quantification of over 182 immune cell phenotypes at a single site. We have applied the protocol to discern minor differences between healthy and patient samples and validated its value for application in immunomonitoring studies. Our protocol is currently used for the characterization of the impact of age and environmental factors on peripheral blood immune phenotypes of >400 donors from the initial MI cohort.