# N-color analysis of biological specimens by Slide Based Cytometry: An important analytical tool in Cytomics.

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There is always a need for new colors in immunology to detect subsets of leukocytes. Detection of minor cellular subsets such as dendritic cells, antigen specific T-cells or circulating stem cells need a minimum of three to four colors. But for further high content characterization additional colors are necessary. In addition, in order to characterize minimal cell populations with specific expression patterns a thousands of individual cells need to be analyzed. This is however not achievable with most of the state of the art technologies. By slide based cytometry instruments like the Laser Scanning Cytometer (LSC, a relatively new fluorescence microscope-based technology), up to six colors are detectable simultaneously. The guiding principle of the instrument is that any specimen has to be immobilized on a microscope slide. This has several advantages: cells are not lost in a fluid stream but are kept on the slide and therefor even minimal specimens as low as 1.000 cells can be analyzed. In addition cells are available for further analyses; such as for the documentation of the morphology, FISH or single cell PCR. We have established an assay for eight-color immunophenotyping of peripheral blood leukocytes (PBLs) by LSC taking 10µl blood. The specimen is prepared according to routine flow cytometry protocols with a set of antibodies but the cells are fixed on microscope slides. With our adaptation any combination of the following dyes can be used FITC, PE, PE/Cy5, PE/Cy7, APC and APC/Cy7. Using different antibody combinations minor subsets can be quantified and morphologically documented. This assays further improves the microanalytic immunophenotyping of PBLs enhancing both, the quantity and resolution of the data.
Due to the immobilization of cells on the slide the information density is further increasable by combining different techniques:

1. Sequential photobleaching: Following simultaneous labeling of cells with dyes having similar emission spectra but different photostability (e.g. PE, Alexa532, quantum dot 585) the same color may be used to distinguish different cell compounds. By sequential photobleaching, change of filters and subsequent merging of the data the number of simultaneously measurable “colors” is substantially increased.
2. Sequential restaining: The immobilized cells may be restained with markers carrying the identical dyes as the initial labels. When the cells data before and after cytometric analysis are combined into one file the data obtained in the same fluorescence channel before and after staining are regarded as two different (virtual) colors. This procedure can be repeated several times.

By including only these two simple techniques, Slide Based Cytometry enables for the analysis for 17 or more colors per individual cell in a high content and high throughput fashion. The information density may be further increased by implementing FLIM, FRET or other technologies. Therefore, Slide Based Cytometry is an important tool for detailed analysis of hypocellular specimens and a state-of-the art technology for Cytomics analysis of biological specimens.

Keywords: Immunophenotyping, Laser Scanning Cytometry (LSC), CD-Antigens

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