

The first fluorescence-based flow cytometry device was developed in 1968 by Wolfgang Göhde from the University of Münster. This technique is now widely used for counting and examining cells and other microscopic particles by suspending them in a stream of fluid and passing them in front of an electronic detection system. It allows for the rapid multiparametric analysis of physical and chemical characteristics, and also for the separation of cells based on those characteristics. Applications of cytometry include diagnosis (e.g. tumor immunology, transplantation, hematology, sperm sorting) and research (e.g. characterization and separation of immune and other cells). This talk will therefore benefit both clinicians and researchers interested in the identification, characterization, and separation of cells and other particles.

In flow cytometry, microscopic particles are either labeled with fluorescent markers such as fluorophore-conjugated antibodies specific for proteins present on the surface or inside cells, express fluorescent markers, or are loaded with dyes. A beam of light of a single wavelength, generated by a laser, is directed on a stream of fluid containing the particles of interest. Detectors are aimed at the point where the stream passes through the light beam. The positioning of the detectors allows for the measurement of particle volume and the presence of granules and/or fluorescent labels. The data generated by flow cytometry can be plotted in one, two, or three dimensions. The different regions on these plots can be sequentially separated, or "gated" based on fluorescence intensity. Examples of such data analysis will be presented during the talk.

Over the last decades, the field has known a tremendous increase in capabilities with commercial multi-laser instruments allowing for the detection of over 20 different markers in single cells. The fluorescent-based technology was however reaching its limits until recently, with the development of mass cytometry. Mass cytometry uses atomic mass spectrometry to detect markers in single cells. Antibodies, DNA intercalators, and nucleic acid probes are labeled with metal tags. Cells are stained with those antibodies and probes, as in conventional flow cytometry, and introduced individually into an inductively-coupled plasma where the cells are atomized and ionized. The atomic ions are extracted into the ion optics and time-of-flight regions where they are separated by mass and counted. The elemental signature of each cell includes the metal tags introduced with the antibodies and probes and is analyzed separately. The presence of the metal tag indicates that the probe found and bound the target marker. The intensity of the signal is directly proportional to the number of probes bound per cell.

Mass cytometry presents several advantages over classical flow cytometry, especially for uses in combination with MRI. (1) The periodic table of elements contains 13 lanthanides that are available in 37 enriched isotopes, thus permitting assays with up to 37 parameters using the lanthanides alone. An additional 30 isotopes of the noble metals can be used to create a panel with 67 parameters. Other isotopes are available to mass cytometry, though different chelation (or other binding) chemistry is required. Taken together, all the isotopes detectable by mass cytometry add up to 100. This means that up to 100 different markers can potentially be detected in individual cells using this technology. For scientists using lanthanides as contrast agents in MRI, mass cytometry presents a significant advantage. One could envision injecting the contrast agent for MRI, following by tissue collection for identification and characterization of single cells labeled with the lanthanide. (2) Conventional cytometry using fluorophores is limited by overlap of emission spectra of the different fluorescent labels. This requires compensation before any multi-color analysis is possible and careful choice of fluorescent tags during experimental design. In contrast, mass cytometry provides at least three orders of magnitude resolution between adjacent metal isotopes, thus eliminating the need for compensation. In addition, the detection sensitivity is practically the same across all metals measured. These features mean that the selection of labels used in an experiment is arbitrary and that each tag can be selected independent from the characteristics of other tags, making complex multi-

marker experiments easier to design error-free. (3) Only element tags can be detected by atomic mass spectrometry. Since the metal tags used are chosen from rare elements whose natural concentration in biological samples is below the detection limit, unlabeled cells remain invisible to the mass cytometer. This feature prevents any auto-fluorescence-like effects that make classical flow cytometry sometimes difficult to use.

At the end of this talk, attendees will have learnt the basics of flow and mass cytometry. This will provide them with the tools necessary to select these methods for extending their work to single-cell analysis.