IRTG 914 - Advanced Methods Course – Winterterm 2015/16

Fluorescence activated cell sorting (FACS)

Every cell in our body has a specific phenotype which is dependent on its individual function. Fluorescence-activated cell sorting is an important tool to purify cells based on these phenotypical differences. The properties measured include cell size, granularity and expression of surface proteins.
Within the flow cytometer, single cells are transported to a laser unit within a fluid stream. When the cell passes the laser, it emits light. The light passes through optical filters which direct the light to the appropriate detectors. These optical signals are then converted into electronic signals which can be processed by the computer. By this means, cells can be captured and collected for further analysis.

As mentioned above, the purification of single cell types can be accomplished by specific characteristics of the cells. Cell size and granularity can be measured using the deflected light that is scattered when the cell passes the laser. For example, major leukocyte subtypes like lymphocytes and myeloid cells can be differentiated by their size and granularity. However, to detect specific cellular subpopulations within a mixed population of cells, you can make use of their individual protein expression levels. A protein which is uniquely expressed by a specific cell type can be tagged with an antibody coupled to a fluorescent dye. Combining different fluorochrome-conjugated antibodies with the information about size and granularity allows for highly sensitive purification of cellular subtypes.

The isolation of leukocyte subpopulations represents an important tool in research since it allows for molecular and functional studies even of tiny cell subpopulations. Therefore, the aim of this course is to give an overview about the methodology of fluorescent activated cell sorting and to provide insight into practical aspects of sample preparation and cell isolation.

Literature:

- Arnold and Lannigan, Curr Protoc Cytom. 2010; Practical Issues in High-Speed Cell Sorting
- Ibrahim and van den Engh, Curr Opin Biotechnol. 2003; High speed cell sorting: fundamentals and recent advances.