

Establishment of routine methods for the examination of the immune system of harbour seals (*Phoca vitulina*) (flow cytometry / health assessment)

Projectdata

Project leader:

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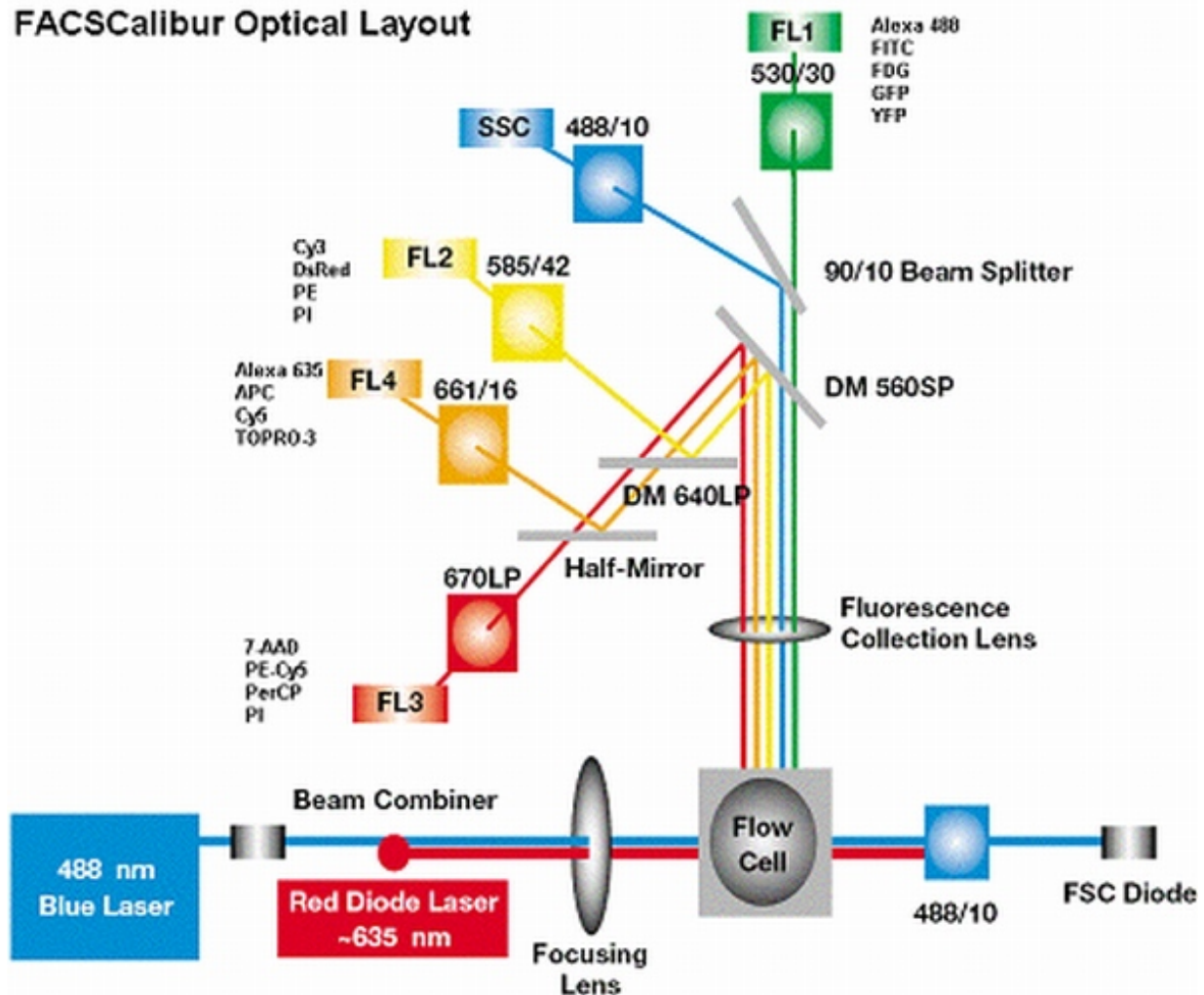
The flow cytometer used in the present project is a FACS Calibur by Becton Dickinson Biosciences (San José, CA, USA). The FACS Calibur is a four-color dual laser, desktop analysis device (Fig. 1). It is a flow cytometer easy to handle, but at the same time is able to use prevalent coloring antibodies due to two laser lines. By an additional diode-laser fluorochromes can be used, which are not detectable by the common 488nm (blue) but lie at 635nm (red).

In this project the flow cytometer is used primarily for the phagocytosis tests but has been and will be used for other tests as well. Only the blue-green excitation light (488nm argon-ion laser) has been necessary for all applications.

Phagocytosis:

Phagocytosis is the most important and initial defense against many pathogens in mammals and humans. In general, it is the ability of cells to ingest extracellular macromolecules. This can happen in different ways. In order to verify the efficiency of the immune system it is possible to examine the functionality of the phagocytes. The phagocytosis test enables a quantitative analysis of phagocytic function of neutrophils and monocytes in a blood sample using either immunofluorescence microscopy or flow cytometry. Either assay can measure the overall percentage of monocytes and neutrophils showing phagocytosis (ingestion of one or more bacteria per cell) and the individual cellular phagocytic activity (relative number of bacteria per cell). Acquired immunodeficiencies can result in increased susceptibility to infection, some being due to defective neutrophil phagocytosis.

FACSCalibur Optical Layout



Sketch of a FACS Calibur with commonly used fluorochromes.

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