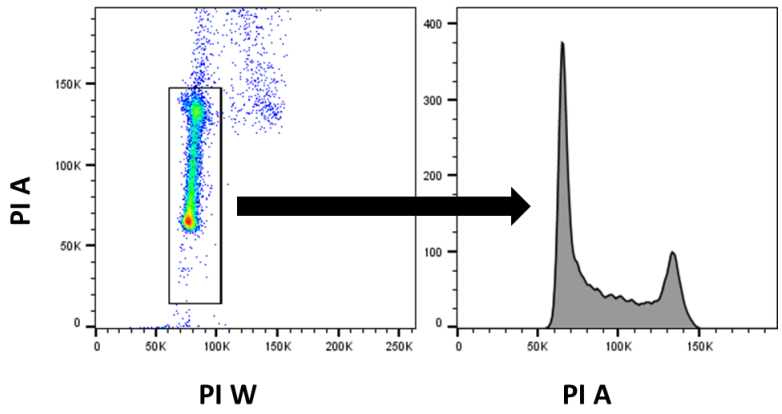
Cell Cycle Analysis

The cell cycle profile of a sample can be determined by staining the DNA with a fluorescent dye and measuring its intensity. From 5 different cell cycle-phases only 3 can be distinguished by Flow Cytometry (G1, S, G2). A variety of staining protocols can be adapted for different sample types, but the general analysis remains the same.



Some DNA dyes do not stain live cells. The sample must be fixed and permeabilized to allow the dye to enter the cells.

- Propidium Iodide (PI) – Blue/Green lasers, also stains RNA
- DAPI – UV/Violet lasers
- Some DNA dyes are membrane-permeable and can be used to stain live, intact cells.
- Hoechst 33342 – UV/Violet lasers
- DRAQ5 – Red lasers

Tips:

Count the cells and calculate the cell concentration

Use the same number of cells in all the samples

PI cell cycle staining protocol:

1. Harvest cells and resuspend in PBS
2. Add cold ethanol, dropwise, to a final concentration of 70%
3. Fix on ice for at least two hours
4. Wash in PBS and resuspend in staining buffer (PBS with 100 µg/mL RNaseA, 50 µg/mL Propidium Iodide,and optionally 0.1% Triton X-100). During the staining sample should be protected from light
5. Incubate overnight at 4°C
6. Acquire data on a flow cytometer