Flow Post-its
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Cell Cycle by Flow Cytometry

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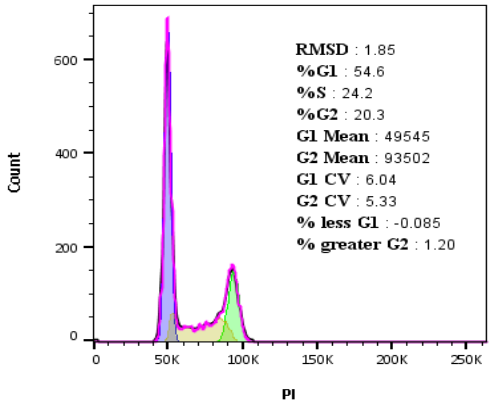
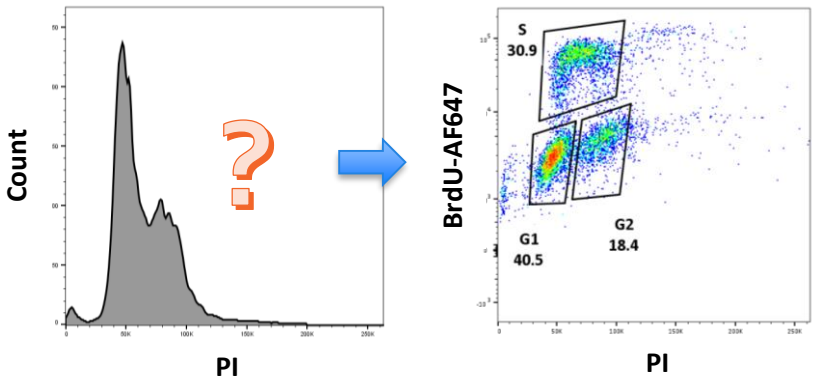
Studying **Cell cycle** by Flow Cytometry can be performed by staining cells or nuclei with **fluorescent DNA markers** or **nucleoside analogs** and measuring the signal output. By using DNA dyes that bind stoichiometrically we can assess DNA ploidy level, cell cycle stage, the presence of apoptotic cells and performance of drugs for treatment of disease states .

Cell cycle analysis can be carried out with **live or fixed cells**. When fixing cells for cell cycle analysis, alcohols (e.g. 70% EtOH) are suggested over crosslinking fixatives (e.g. aldehydes) for cell cycle analysis. Propidium Iodide (**PI**) and 4',6-diamidino-2-phenylindole (**DAPI**) are commonly used dyes. If using fluorochromes that bind both DNA and RNA (such as PI), then inclusion of RNAse A is required.

Alternatively, live cell cycle analysis can be done with dyes that cross membranes. When this is done, an efflux pump blocker may be required. If using **Hoechst 33342** consider adding dye DiOC5(3) or Verapamil. **DRAQ5** is another supravital dye available, but care should be taken as it can be cytotoxic for cultured cells. There are also a variety of **Vybrant DyeCycle** fluorochromes available which can be used across a variety of instrument configurations.





Addition of a nucleoside analogs, such as a fluorescently tagged **BrdU** or **EdU**, allow a more accurate evaluation of the different cell cycle phases.

Experimental Tips and Tricks

- Use the same number of cells per sample and concentrate appropriately to run at a low flow rate to ensure best resolution. $1x10^6$ cells/mL is a good start.
- Optimize dye concentration for best resolution of each cell cycle stage.
- Before adding fixative, ensure cells are in a good single cell suspension.
- For univariate cell cycle data analysis, mathematical modeling approaches available in various analysis programs are suggested. Manual gating is not advised.
- **Data acquisition** and **analysis** of all cell cycle data should be done with the DNA fluorescent parameter on a linear scale.

Useful References

Darzynkiewicz, Z. 2011. doi: 10.1002/0471142956.cy0702s56
Darzynkiewicz, Z. and Juan, G. 2001. doi: 10.1002/0471142956.cy0705s00