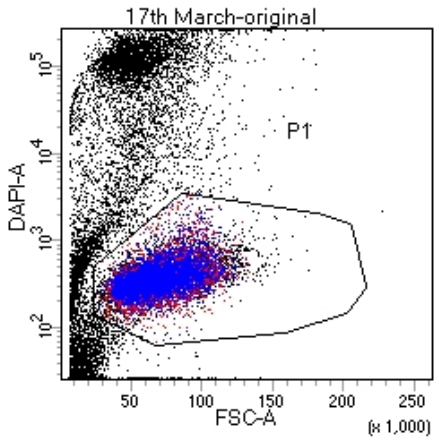
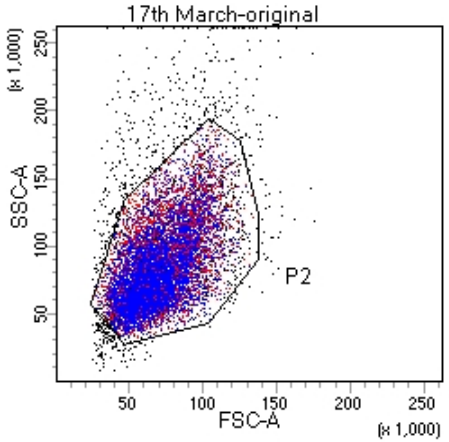
PROTOCOL PLOTS & GATING

Basic template for good cytometry data

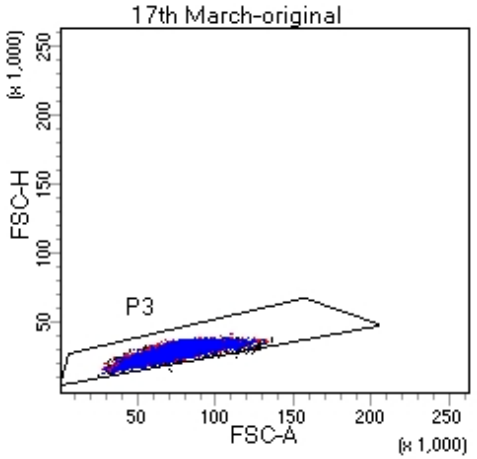
1. Viable Cells



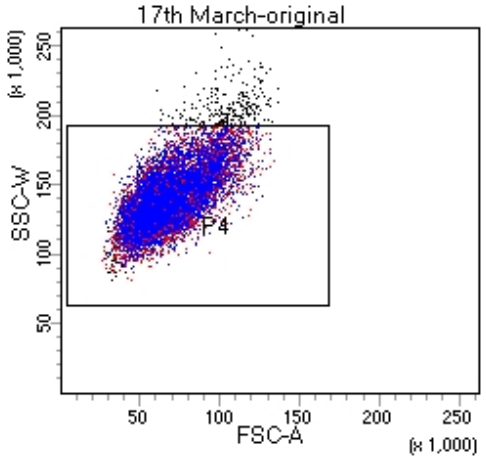
2. Remove Debris & Unwanted Cells



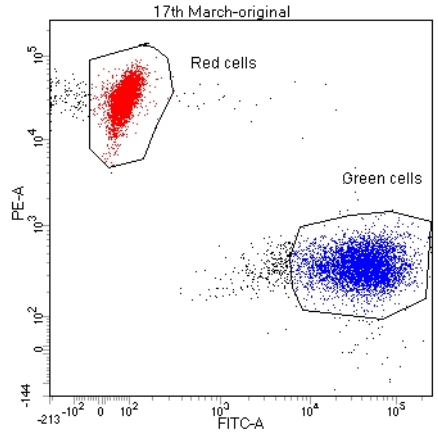
3. Single Cells I



4. Single Cells II



5. Fluorescent Markers



1. Add a viability dye to avoid including dead cells and creating false positives. The viable population should be clearly observed such that it can be gated confidently.

2. Put a gate around the cells required based on size. If not known draw a generous gate, removing debris in the bottom left corner. Large & more complex cells will be high SSC & high FSC. Smaller, rounded cells will be low SSC & low FSC.

3. Gate single cells. Remember to select H,W & A for the scatter channels in the **parameters** tab.

4. Use 2 single cell plots with different parameters (here using SSC-width). Different types of cells will have a clearer population in a particular combination of H,W,A of FSC & SSC

5. Use a negative control and FMO´s in order to be confident of the placement of gates for positive populations. Use log scale for the fluorescent markers and adjust voltages* so that all populations can be clearly seen.

6. Ensure each gate is derived from the previous one.

*another slide will show you how to do this

Gating Order

Tube: original

Population	#Events	%Parent	%Total
All Events	21,792	####	100.0
P1	10,450	48.0	48.0
P2	9,868	94.4	45.3
P3	9,752	98.8	44.8
P4	9,514	97.6	43.7
Red cells	4,740	49.8	21.8
Green cells	4,305	45.2	19.8