PROTOCOL CELL CYCLE 2 - SAMPLE AQUISITION

Set up an instrument to record data

Example: Propidium Iodide (PI) using the YG laser in the PE-Texas Red PMT

To Remove Cell Doublets

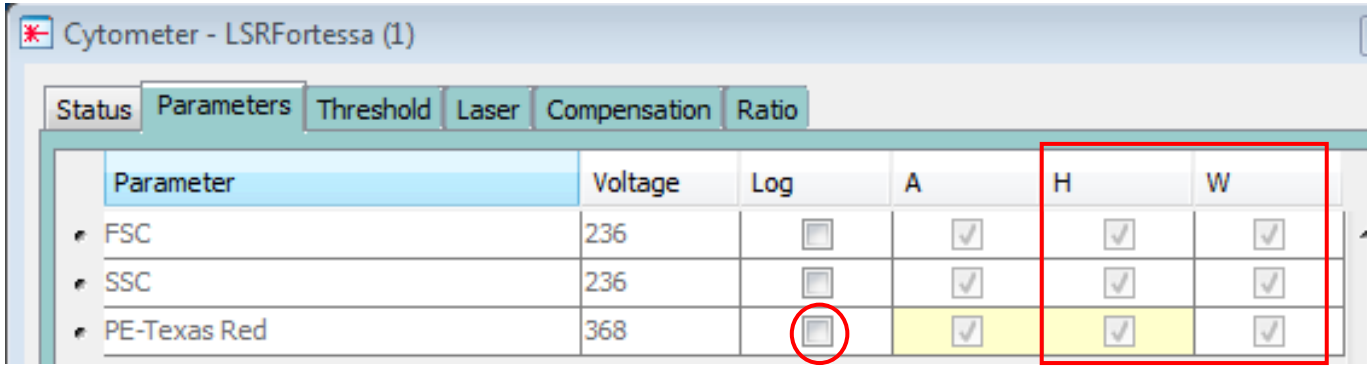
In the parameters tab select height and width signal measurement for the scatter channels

To Remove Nuclei Doublets

Select height and width parameters for the cell cycle dye

Select Linear Scale

Deselect log for the cell cycle dye channel



Plots & Gating

Set up 4 dot plots and a histogram in the following order.

1. Area vs Height of the dye. (Some cytometers use Area vs Width). Selects **single stained nuclei**.
2. Remove debris
3. Remove cell doublets
4. Include an extra doublet plot to ensure only single cells are selected
5. Use the Area signal measurement for the dye.

Important: run the samples in LOW pressure mode to improve the resolution of the histogram.

Adjust voltage so that the G1 peak is in line with the 50K channel for optimum sensitivity & to eliminate the small inconsistencies of staining between samples