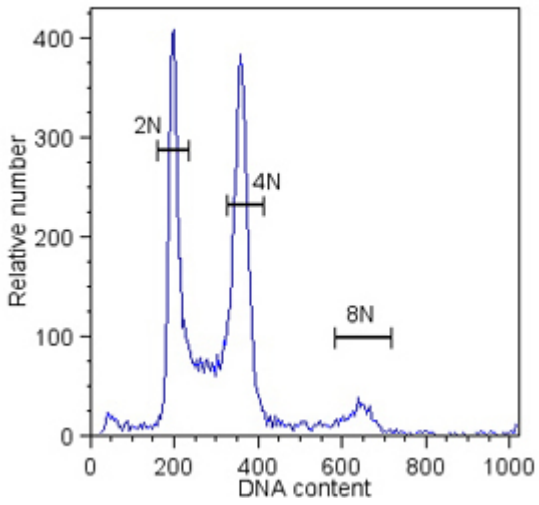
Common Applications

Cell Cycle

One of the most common applications of flow cytometry is measurement of the DNA content of cells. In this way different treatments can be assessed for their effect on cell cycle distribution: for example, treatment with a drug or transfection with a gene of interest. The technique can easily be applied to both mammalian and yeast cells, although due to their much smaller size and DNA content, yeasts show less defined phases of the cell cycle.

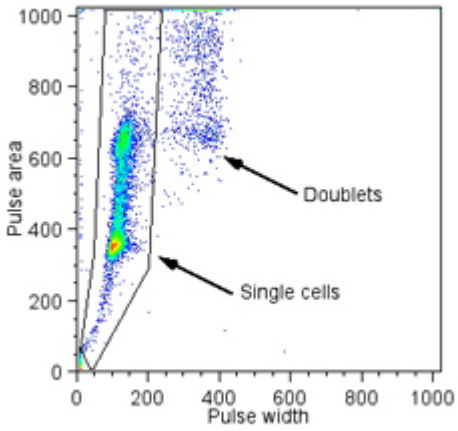
There are many different fluorophores that can be used to determine DNA content, each having differing excitation and emission wavelengths. This makes it possible to combine cell cycle analysis with many other different fluorophores. A number of the more common dyes used to quantify DNA content are outlined here. Some dyes, such as DAPI, are specific to DNA and do not bind to RNA, whereas others, such as propidium iodide, bind to both DNA and RNA and require the removal of RNA (using RNase) before an accurate DNA profile can be obtained. Most DNA binding dyes are impermeable to live cells (Hoechst 33342 being the exception), which must be permiablised before DNA content can be measured. The most common method of permiablisation is alcohol fixation, which has the added advantage of allowing storage of fixed samples for many weeks before staining. However, alcohol fixation can have a deleterious effect on other cellular characteristics (eg antigen integrity). To overcome these effects, saponin may be used, which allows the DNA dye to enter 'live' cells, making it a more suitable technique in some instances.

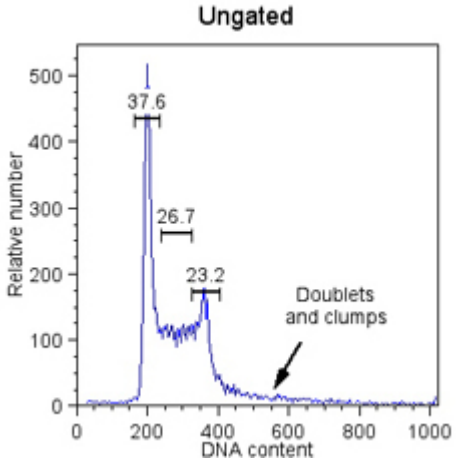
When using flow cytometry to assess DNA content, the fluorescence data (FL-H) is acquired with linear amplification and plotted on a histogram from channel number 0 to 1023. It is common practice to set the G1 peak to around channel 200, which allows good resolution of the G1, S and G2/M phases while still allowing endoreduplicated/multi-nucleated cells (>4N) to fit on the scale. The quality of cell cycle data can be assessed by looking at the co-efficient of variation (CV) of the G1 peak. The lower the CV the better quality the data but generally a CV of <6% represents acceptable data.

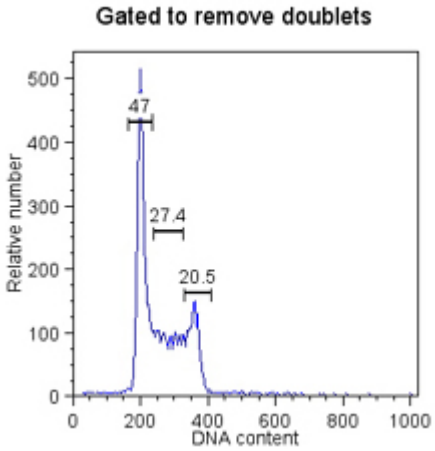


Another benefit of flow cytometry as a means of assessing cell cycle distribution is its ability to exclude doublets from the analysis. Doublets are a problem because a doublet of two G1 cells will have the same DNA content as a single G2/M cell. 'Doublet discrimination' is a process

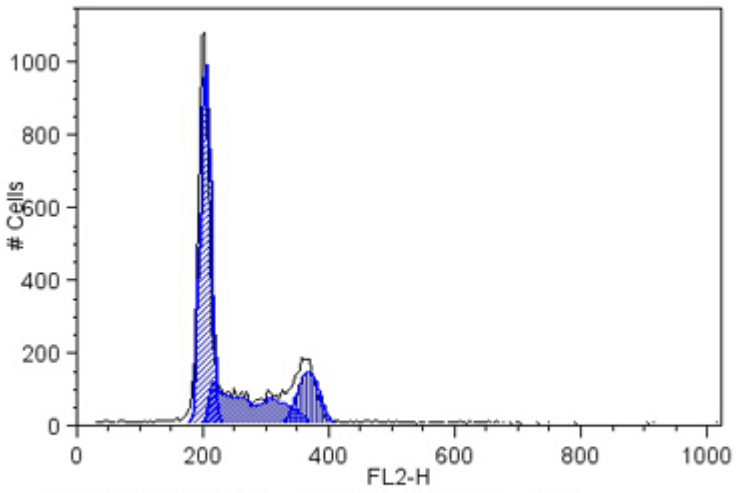
whereby the area (FL-A) of the fluorescence light pulse is plotted against the width (FL-W). Doublets will have greater pulse width than a single cell, as they take longer to pass through the laser beam, and therefore can be excluded from the analysis.







When the data has been acquired, the number of cells within the different cell cycle compartments can be assessed. This can be achieved either by manually placing markers or by performing software analysis, in which mathematical algorithms are applied to the data to give values.



Watson Pragmatic: %G1 = 51.4; %S = 28.5; %G2 = 16.5.
Cell Cycle
H2
Event Count: 10027