Appendix I

Data extraction for a systems biology approach has to be carried out in a particular manner to allow the achievement of a systematic analysis of the experimental cytometry data. The data has to be extracted in a regimented manner that allows it to meet the needs of a systems biology approach. Below is a description of the data processing that occurred in order to derive the proportions of cells in G_1/G_0, S and G_2/M from a population of cells using flow cytometric analysis that we can use in our mathematical model. The method used was carried out as Nicoletti *et al*, 1991 and in addition to allowing the identification of the proportions of cells in G_1/G_0, S and G_2/M, a pre-G_1 fraction is also identified, which is representative of cell degradation products, 'dead' cells, which may or may not be apoptotic, mechanically damaged cells, cells with reduced chromosome number and isolated apoptotic bodies. Apoptosis can also result in the complete fragmentation of the nucleus and cytoplasm and when staining of the cells with a hypotonic buffer, several individual nuclear fractions can be produced from a single apoptotic cell and can therefore result in the over-estimation of the proportion of apoptotic cells. Shown here is the derivation of the proportion of cells in G_1/G_0, S and G_2/M. The cells were harvested as in section 2.2.3 and incubated in hypotonic fluorochrome solution as described in section 2.2.7.1. The flow cytometer was instructed to acquire the data as follows:

1. A dot plot of FSC-H versus SSC-H allows us to gain an estimation of the viable fraction.
2. A dot plot of FL3-lin versus AUX from the viable cells that have been gated in (1) allows us to 'pull out' the single cells away from the unwanted doublets.
3. A dot plot of FSC-H versus FL3-Lin of the viable cells allows the further gate out debris.
4. A histogram of FL3-lin of the cells gated in (1) and (2) is used to determine the proportion of cells in G_1/G_0, S and G_2/M. To do this, a fitting algorithm is used based on Watson *et al*, 1987.

In carrying out the above procedure on each set of data and placing gates (1) and (2), we can gate out the cell debris and doublets and therefore allow the histogram of the cell cycle to be 'clean'. Markers allow us to segment the histogram and are placed to allow manual estimations of the proportions of cells in G_1/G_0, S and G_2/M. In using manual estimations, objectivity is difficult to achieve and therefore rules for the segmenting of the histograms needs to be in place. An example of the data extraction used in this study is shown in appendix II.