Application **Note**

AppliedCytometry
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VenturiOne™ Cell Cycle Analysis

Introduction

The DNA content of cells varies in relation to the distinct phase of the cell cycle they are currently in. By staining the cells with a DNA-binding fluorescent dye, such as propidium iodide, the different stages of the cell cycle can be analysed by flow cytometry (Figure 1).

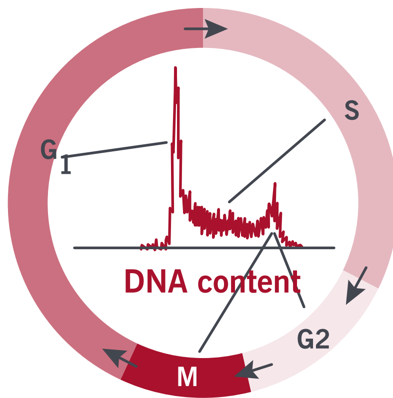

Figure 1. The relationship between the DNA histogram and the cell cycle.

Frequently, an investigator wants to know the percentage of cells in each phase of the cell cycle particularly in the field of cancer research. Here the effects of cytotoxic drug, radiation and other cancer treatments can be observed.

During DNA analysis a problem can arise due to the overlap of cells in G1 and those in early S phase and the overlap between G2/M and late S phase (Figure 2).

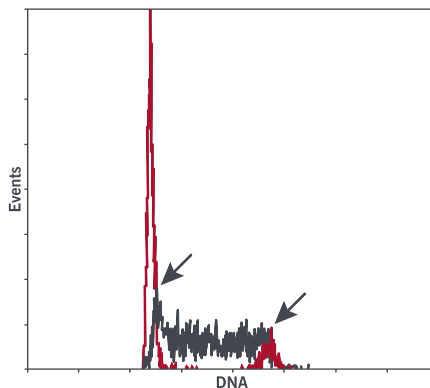

Figure 2. The overlap between S phase, G1 and G2/M phases of the cell cycle. The cells in S phase were labelled with bromodeoxyuridine, which allowed the DNA histograms of G1, S and G2/M phases to be separated. The three histograms were then superimposed.

To overcome this problem routinely the phases of the cell cycle are separated by modelling the DNA histogram using a computer program. Several different modelling approaches can be used. One such approach is the modified Watson 'pragmatic' algorithm which has been employed in the VenturiOne™ cell cycle analysis feature. To follow is a description of the application of the Watson modelling in VenturiOne™ to overcome the overlap between S phase, G1 and G2/M phases of the cell cycle.

Experimental procedures

The cells shown in Figure 3 were fixed in 70% ice-cold ethanol, in which they were stored at -20°C. For analysis, they were resuspended in PBS with propidium iodide (PI) at 20μg/ml and RNase at 1 mg/ml. For this and other staining protocols see Ormerod, 2000, and Current Protocols in Cytometry, 2007.

Analysis

The model assumes that G1 and G2/M are normally distributed and fits Gaussian curves to them, returning the peak channel number and the coefficient of variation (CV). It then uses these values to construct the regions of S phase which overlap either with G1 or G2/M, using curves based on the cumulative error function (erf). The model uses the unmodified experimental values for the centre of S phase. It is described in more detail by Ormerod et al., 1987.

When the cell cycle option is activated in the VenturiOne™ software, a modified Watson algorithm of the cell cycle analysis is automatically applied to the selected histogram plot. The three phases associated with the DNA cell cycle are distinguished by colour. Red denotes the G0/G1 phase, blue denotes the S phase and green denotes the G2/M phase.

There are also user adjustable markers present on the cell cycle plots. A red marker is positioned at the estimated central point of the G0/G1 phase and a green marker is positioned at the estimated central point of the G2/M phase. Additional blue markers are displayed on the plots to set noise thresholds. Events that occur above or below these thresholds are presented on the plots but not analysed by the software.

Some results using the VenturiOne™ software are shown below. In Figure 3A, a large proportion of cells are in G0/G1 phase as indicated by a large red peak whereas in Figure 3B, the majority of cells are in G2/M phase indicated by a large green peak.

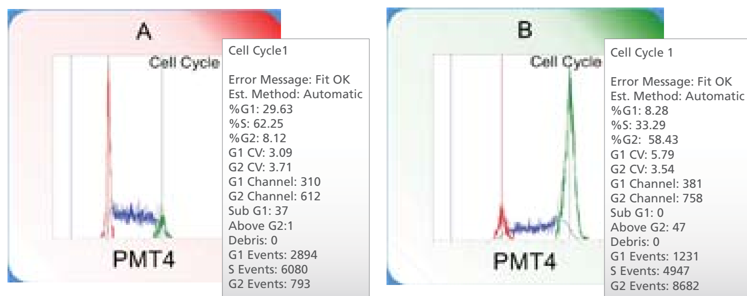

Figure 3. Cell cycle analysis of (A) a murine leukaemic cell line, (B) the cells 48 hours after incubation with a cytotoxic drug.

Discussion

Using any program, the goodness of fit will depend on the quality of the DNA histogram. As in the results above, an error message is reported by the cell cycle analysis indicating whether the algorithm experienced any problems. Here the message is Fit OK displaying good G1 and G2 CV's. CV's across the G1 and G2/M peaks should be =<5%. Histograms with CVs >8% should be rejected.

There will be occasions when the program clearly has not fitted the histogram correctly. The most likely source of the problem is fitting G2 and the S/G2 overlap due to a lot of noise. This is easily detected as the CV G1 and G2/M peaks are vastly out of line. You should experiment by changing the starting position of the G2 peak.

The Watson model used in the VenturiOne™ software is a simple and robust method as it requires the assumption that the data are normally distributed and a recognisable G1 peak; this may be vestigial although from referenced literature, rarely absent. Based on these assumptions, VenturiOne™ can produce fast and simple cell cycle analysis automatically therefore reducing the need for user intervention. As seen in the results, it can also be used to show differences between typical cell lines and those treated with cytotoxic drugs without having to take anything else into account.

Note

This program is for research use only.

References

Current Protocols in Cytometry, 2007. John Wiley & Sons, Inc.

Ormerod, M.G. (2000). *Flow Cytometry. A Practical Approach.* Ormerod, M.G. (ed). 3rd edition. Oxford University Press, Oxford. 83-97.

Ormerod, M.G., Payne, A.W.R. and Watson, J.V. (1987) *Improved program for the analysis of DNA histograms*. Cytometry **8**: 637-641.

Watson, J.V., Chambers, S.H. and Smith, P.J. (1987) *A pragmatic approach to the analysis of DNA histograms with a definable G1 peak*. Cytometry **8**: 1-8.

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