A Novel Cell Cycle Analysis Protocol Utilizing High Content Screening in Adherent Cell Line HS-27

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Analyzing how drugs affect the cell cycle requires cell cycle analysis. An approach using flow cytometry is limiting as the only parameter that can be collected is intensity. In order to obtain additional information, including the localization of proteins, High Content Screening (HCS) must be used. The purpose of this study was to improve the quality of DNA content histograms taken from HCS and to model the data as distinct cell cycle events using computer software. HS27 cells were seeded at various densities to optimize cellular density. Cells were then stained using two different dyes at various concentrations to optimize concentration and minimize photo-bleaching. HCS was then used to analyze the DNA content in the cells. Raw data was collected by analyzing the images on ImageJ, which was then transferred over into FlowJo, where samples were gated, and later analyzed in Modfit. DNA histograms obtained from the HCS resembled data from flow cytometry. The CoV's, were significantly higher on the HCS than on the flow cytometer. The histograms were sufficient to recognize events in the cell cycle, and the images could be used to observe translocation of cellular structures through multi-channel displays. The cell cycle analysis data taken from HCS was sufficient to recognize cell cycle events, but not sufficient to provide quantification of cell cycle events comparable to flow cytometry. Additional studies to optimize the permeability of dyes in live adherent cells, as well as optimization with other DNA dyes may be undertaken.