

Analysis Regulation of Protein Expression in the Cell Cycle on the EIDAQ™ 100 High Throughput Microscopy System

A measurement of protein expression and correlation to the cell cycle is described, using the EIDAQ™ 100 High Throughput Microscopy system from Q3DM. Integrated fluorescent intensity is used for multiparametric analysis of protein regulation during the cell cycle. For accurate reading/measurement, the EIDAQ™ 100 automatically images populations of cells, defines cell morphology, measures the amount of specific fluorescent signals in each cell. The EIDAQ™ 100 is a novel High Throughput Microscopy (HTM) system that delivers accurate, quantitative, imaging, and analysis of cell populations, at high speeds, directly from slides and microtiter plates.

Background and Significance

The cell cycle is the "program" for cell growth and cell proliferation. There are 4 broad phases of the cell cycle: G1 (and G0), S, G2, and M. A number of proteins regulate and control the cell cycle, these include: cyclins, cyclin-dependent kinases (CDK's), CDK inhibitors, and suppressor genes such as p53. Proteins that are regulated during the cell cycle include the replication machinery itself, chromatin unwinding proteins, damage sensing and repair complexes, cytoskeletal components, and other members of complex regulatory networks, including proteases, kinases and phosphatases that activate, inhibit or sequester transcription factors. Any of these molecules may be a drug target for diseases such as cancer and immune system disorders. The ability to quantify protein expression in individual cells in unperturbed populations, and to correlate this with cell cycle status is a powerful tool in the analysis of cellular responses to potential therapeutic agents.

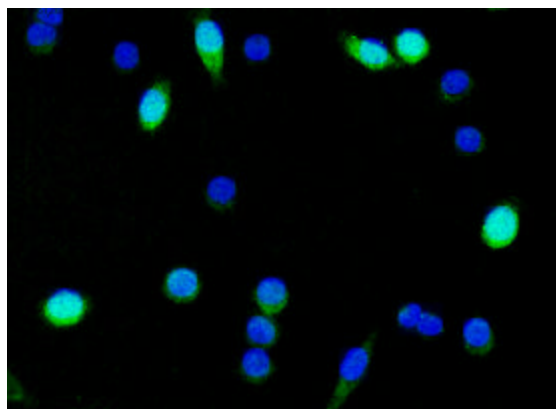


Figure 1 Example 20X 0.5 N.A. fluorescent micrographs of HeLa cells using the EIDAQ 100™ High Throughput Microscopy (HTM) system. CyclinA is detected as a GFP fusion protein (green) and the nucleus is defined by Hoechst 33243 staining (blue)

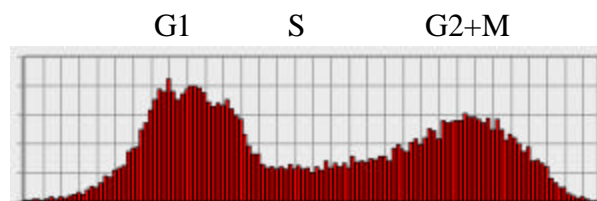
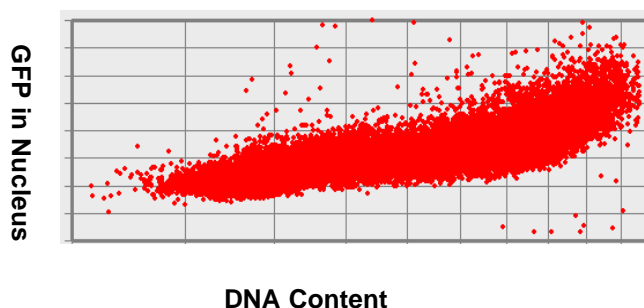


Figure 2 (above) Scatter plot of 12,000 cells with DNA content along the X axis and fluorescent intensity of green signal in the nucleus on the Y axis. **(below)** The histogram of the same cell population is shown with DNA content on the X axis plotted on the same scale as in the scatter plot

APPLICATION NOTE

Experimental Methods

Human cervical carcinoma cells (HeLa) were grown in a monolayer. Simultaneous detection of cyclin A protein and cell cycle stages was observed. Cyclin A expression and DNA content in HeLa cells were detected using fluorescence on the EIDAQ™ 100 system. Cyclin A fluorescence was tracked with GFP-fusion (green emission) while nuclei were detected through Hoechst 33243 (blue emission). Q3DM's proprietary metrics were used to quantify the integrated fluorescent intensity.

Experimental Results

The combination of automated sub-cellular imaging, proprietary image processing and computational geometric methods for cell compartment definition enabled rapid, accurate correlation of cyclin A nuclear expression to the cell cycle. From the DNA content histogram in figure 2, G1, S and G2+M subpopulations may be distinguished and a G2 block is evident. From the scatter plot in which the total integrated intensity of green fluorescence inside the nuclear mask delineated by the Hoechst stain, a steady rise in Cyclin A expression can be seen through S phase, peaking as the DNA content reaches G2. A GFP negative subpopulation with G2 DNA content was observed. Using the gating and montage capabilities of the CytoShop™ software on the EIDAQ™ system, these cells were identified as overlapping nuclei and were thus excluded from analysis as artifacts of cell preparation. No mitotic figures (metaphases or anaphases with G2 DNA content) were seen in this population, which may be due to the observed G2 block.

Conclusion

The EIDAQ™ 100 HTM system is able to quantify protein expression in cell populations with enhanced statistical relevance. The Cyclin A-GFP fusion protein appears in the nucleus at the beginning of S phase and increases to a peak in G2. When used in screening, this assay can identify agents that perturb the cell cycle and elucidate the mechanism of action by simultaneously measuring the expression of cell cycle regulating proteins.

The EIDAQ™ 100 automated High Throughput Microscopy (HTM) system from Q3DM Inc. delivers an unmatched combination of speed, accuracy, and detail to quantitative imaging and analysis of cell populations. The EIDAQ™ 100 is used to accelerate drug discovery, for clinical diagnostics, and in basic research.