Automated Analysis of Stem Cell Growth & Differentiation

Richik N. Ghosh, Vivek C. Abraham, Jeffrey R. Haskins and Paul Sammak

Materials and Methods

In all cases, stem cells cultured in microwells were imaged in multiple fluorescence channels using the Thermofisher Scientific ArrayScan® HCS Reader. A channel is typically associated with a distinct fluorophore, but may also represent a different exposure condition. Images of the same fluorophore may also be used to extract different kinds of cellular measurements in different channels. Different automated image analysis methods (Bioapplications) were used depending on the nature of the cellular measurements required for each case.

Analysis of Pluripotency and Cell Proliferation in Undifferentiated Stem Cells

Human embryonic stem cell colonies were imaged for 4 days on a feeder layer of embryonic mouse fibroblast cells. Embryonic mouse fibroblast cells were used to selectively label cells within these colonies because they are indistinguishable from embryonic mouse fibroblast cells with regard to size, shape, Oct4 expression and mitotic index. Pluripotency was quantitatively assessed within individual colonies as the percentage of cells expressing the Oct4 transcription factor, and cell pluripotency and mitotic index were measured using automated image analysis of undifferentiated stem cells with image-based cellular analysis is expected to enable the large-scale testing of several additional applications. Key assay output features generated by the Compartmental Analysis BioApplication enable large-scale cell biological investigation in the context of intact cell nuclei.

Results and Discussion

Analysis of Pluripotency and Cell Proliferation in Undifferentiated Stem Cells:

A summary of the measurements extracted from undifferentiated stem cell colonies using HCS methods is shown in Table 2, which represents analysis of 24 colonies having a range of characteristics with regard to size, shape, Oct4 expression and mitotic index.

Optical Sectioning of Stem Cell Colonies

The assay labeling strategy for analysis of undifferentiated stem cells is shown in Table 1. The assay strategy for analysis of undifferentiated stem cells is shown in Table 1, which focuses on the following applications of HCS to the study of stem cell biology:

• Analysis of pluripotency and proliferation in undifferentiated stem cells
• Analysis of stem cell differentiation along distinct pathways

Application of the Morphology Explorer BioApplication to the Analysis of Neuronal Differentiation

Analysis of Neuronal Differentiation

The assay labeling strategy for analysis of neuronal differentiation is shown in Table 4. Cells were fixed and stained with the neuronal differentiation markers (Figure 6). Our future goals are to extend this approach to other neuronal populations.

Conclusions

HCS is now being increasingly employed in drug discovery and development to provide additional insight into the context of intact cells, with high spatial and temporal resolution. This successful initial application of HCS to the automated analysis of several key parameters related to stem cell growth and differentiation indicates that it will likely prove to be a powerful approach in this area as well. Our future goals are to apply HCS methods to develop applications of growth and differentiation conditions for a range of stem cell applications.

For more information, please visit www.thermo.com/hcs