Optimizing the high content analysis of cell morphology using multi-dimensional imaging on the Thermo Scientific ArrayScan XTI HCA Reader – Part 1 Stem Cell Colonies

Monica Tomaszewski, Richik Ghosh and Mark Collins
Thermo Fisher Scientific • Pittsburgh, PA USA

Introduction
High Content Analysis (HCA) has become a widely adopted tool in life science research with applications ranging from target validation through drug screening to toxicology and basic cell biology research. High content assays provide benefits over traditional biochemical assays because they analyze single cells using imaging as a detection mode, and therefore, are particularly well suited to complex morphological analyses of cells, cell structures such as neurites, and aggregations of cells in colonies. With the increase in stem cell research, HCA is being applied to complex stem cell morphologies and 2D and 3D microenvironments. These more complex assays pose challenges for image acquisition because the samples are often thicker and more heterogeneous, (e.g., stem cells on a feeder layer rather than a layer of cells on the bottom of a microplate), requiring new tools to analyze these biologies.

The Thermo Scientific™ ArrayScan™ XTI High Content Analysis (HCA) Reader can integrate an innovative confocal microscopy module with powerful imaging and analysis software to provide a variety of multi-dimensional imaging capabilities.

This study demonstrates that biological assay results can be significantly improved by choosing the appropriate image acquisition mode. Integrating these modes into an automated high-content imaging platform extends the benefits of high content into new areas of cell biology.

Methods and Materials
An ArrayScan XTI HCA Reader equipped with integrated confocal microscopy module (CrEST X-light™), and Thermo Scientific™ iDev™ software was used in the following three modes for three separate biologies.

- Confocal microscopy – z-stack acquisition
- Widefield microscopy – z-stack acquisition
- Widefield microscopy – single image acquisition

Each imaging mode was used to acquire images from multiple wells of 96-well plates (Perkin Elmer Packard™ View 96,) containing induced pluripotent stem cell (iPSC) colonies (System Biosciences) on mouse embryonic fibroblast feeder layers that were labeled for nuclei (Hoechst 33342; blue fluorescence), and Oct4 (indirect immunofluorescence with Alexa Fluor™ 488 secondary antibody; green fluorescence) (Antibodies and Hoechst obtained from Life Technologies).

Results
There is a maximum trade-off of approximately 30% in terms of performance for acquisition of confocal image stacks compared to widefield (Table 1). However, this reduction is not that significant for the benefit of improved image quality and signal-to-noise.

Due to the thickness of these colonies, non-specific background from out-of-focus planes was seen in the widefield images leading to less crisp definition of the individual stem cells in the colony (Figure 1). Confocal imaging provided sharper definition of the individual stem cells in the colony by rejecting the non-specific signal and was also able to remove the non-specific haze seen in the Oct4 channel images acquired using widefield and widefield with Z-stack (maximum projection).

Figure 1: Images of a stem cell colony acquired using confocal and widefield imaging modes on the Thermo Scientific ArrayScan XTI HCA Reader.
Although the confocal images appear to have less Oct4 signal, the confocal removes this out-of-plane light, leading to a more precise result. Images clearly show the improvements due to the confocal mode, with the cells in the confocal image more clearly defined (blue nuclei) and the center of the colony appearing more blue due to the non-specific Oct4 signal being rejected (the observed green haze in the widefield images (Figure 2).

These stem cell colony images were then analyzed with the iDev software and Thermo Scientific™ Compartmental Analysis (CA) BioApplication. Green image overlays outline the cells identified in the image based on the Hoechst labeling (Figure 2). The red overlay denotes the cells that were positively identified in the image based on the Hoechst nuclear labeling and also were positive for Oct4. Even though the colony imaged by confocal microscopy looked more blue in the center, fewer cells overall were identified in the widefield image, resulting in the overlay images showing more Oct4 positive cells in the confocal image versus the widefield images.

The CA BioApplication reports a variety of measurements on the image from the stem cell colony (Table 2). A comparison of cell number of Oct4 positive cells and the percent of Oct4 positive cells support what was observed in the overlay image (Figure 2) where more cells and Oct4 positive cells were detected for the confocal image.

The measurement data clearly demonstrate that the confocal imaging mode provides significant improvements for analyzing stem cell colonies, resulting in improved cell counts and more accurate detection of Oct4+ cells throughout the thick 3D structure of the stem cell colony.

**Conclusion**

Characterization of stem cells during growth and differentiation is becoming increasingly important as researchers seek to understand the underlying mechanisms of pluripotency and differentiation. HCA, with its automated, multi-parameter, quantitative approach, offers the potential to investigate stem cell biology with greater precision and throughput than traditional manual microscopy. However, as the results demonstrate, stem cell colonies offer some imaging challenges, which can be overcome with confocal imaging, leading to more precise and biologically relevant data. The ArrayScan XTI HCA Reader with integrated confocal offers a flexible and highly productive platform for stem cell research.

**References**