HIGH CONTENT SCREENING: UNDERSTANDING CELLULAR PATHWAY

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ABSTRACT

High content screening (HCS) is the convergence between cell-based assays, high-resolution fluorescence imaging, phase-contrast imaging of fixed- or live-cell assays, tissues and small organisms. It has been widely adopted in the pharmaceutical and biotech industries for target identification and validation and as secondary screens to reveal potential toxicities or to elucidate a drug's mechanism of action. By using the ImageXpress® Micro XLS System HCS, the complex network of key players controlling proliferation and apoptosis can be reduced to several sentinel markers for analysis. Cell proliferation and apoptosis are two key areas in cell biology and drug discovery research. Understanding the signaling pathways in cell proliferation and apoptosis is important for new therapeutic discovery because the imbalance between these two events is predominant in the progression of many human diseases, including cancer. The DNA binding dye DAPI is used to determine the nuclear size and nuclear morphology as well as cell cycle phases by DNA content. Images together with MetaXpress® analysis results provide a convenient and easy to use solution to high volume image management. In particular, HCS platform is beginning to have an important impact on early drug discovery, basic research in systems cell biology, and is expected to play a role in personalized medicine or revealing off-target drug effects.

INTRODUCTION

Cellular research traditionally involves analysis techniques such as biochemical assays, microscopy, Western blotting, and flow cytometry (Abraham et al., 2008). Recent advances in high-throughput automated microscopy, now commonly referred to as high-content screening (HCS), combined automated microscopy with image analysis approaches to simultaneously quantify multiple phenotypic and/or functional parameters in biological systems (Giuliano et al., 2003).

Objectives:

To evaluate cellular assays including cell signaling, toxicology, RNAi knockdown, cell differentiation and morphology, cell cycle, neurology, protein trafficking, and receptor activation.

Features:

- **Sample format flexibility**
- **Wide-field CMOS camera**
- **1x – 100x objectives**
- **Up to 5 fluorescent filters**
- **Transmitted light (phase contrast)**
- **Compatible for 6- to 1536-well plates and slides**
- **High-speed laser and image-based autofocus**
- **Solid state light source**
- **Automation friendly**

Figure 1: ImageXpress Micro XLS System

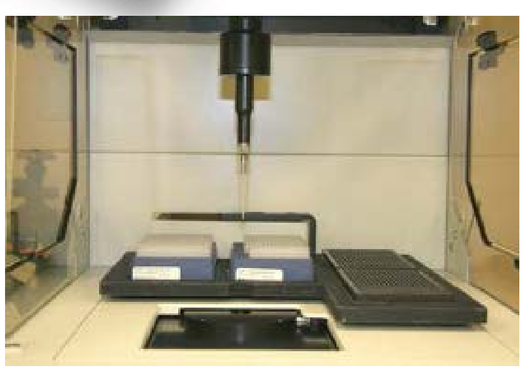
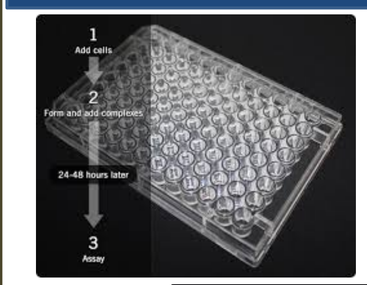


Figure 2: Fluidics option installed in the system

METHODOLOGY



Cells preparation

Load plate/slide

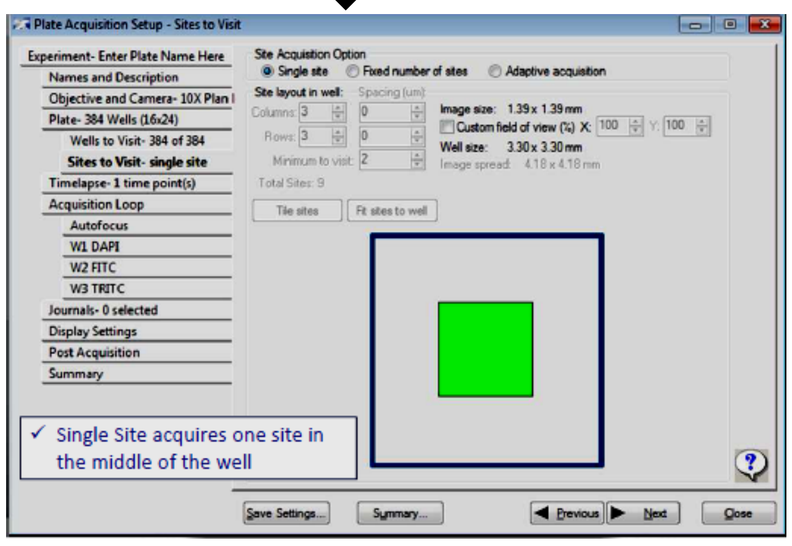
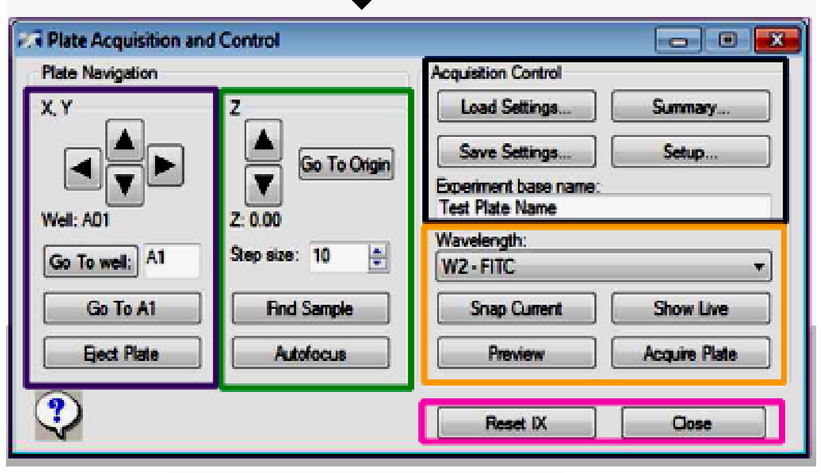


Plate acquisition setup



Capture image

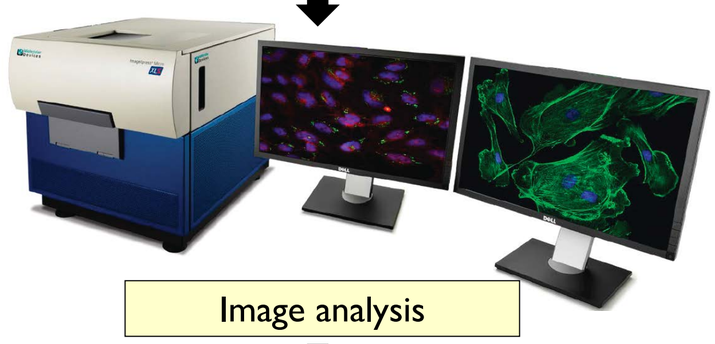
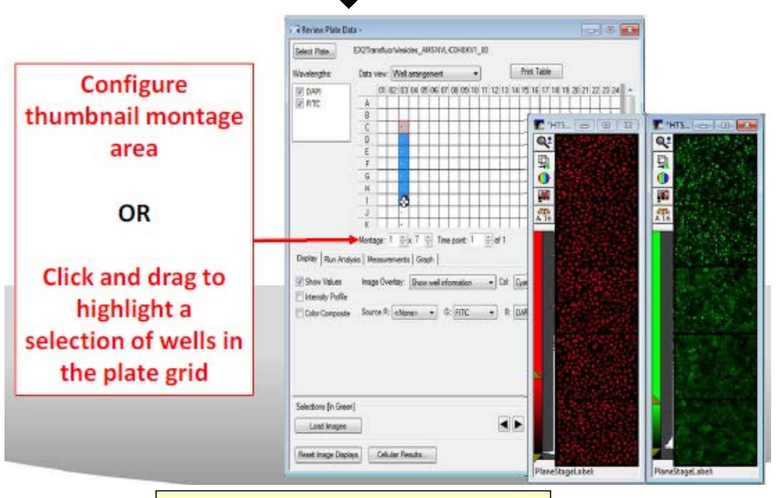


Image analysis



Review results

RESULTS AND DISCUSSION

HCS can be used to screen a variety of models including cells (live or fixed), yeast, virus, bacteria, tissue, 3D culture models, and whole organisms such as zebrafish.

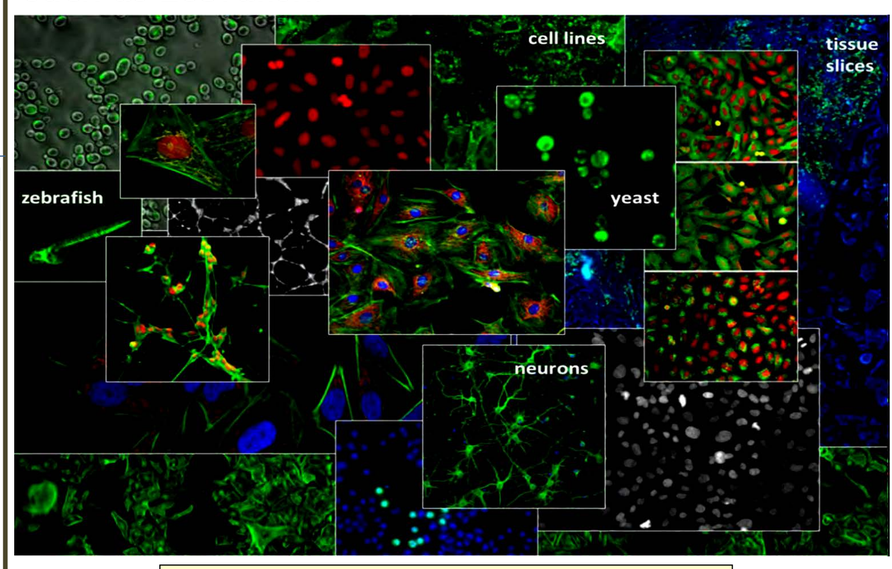


Figure 2: Digital confocal imaging.

The differentially conditioned cell lines exhibit distinct phenotypes. Morphological differences for the cells include larger nuclei and more extensive mitochondrial networks. Such data sets can often provide a reliable "fingerprint" of the action of a particular compound (Figure 3).

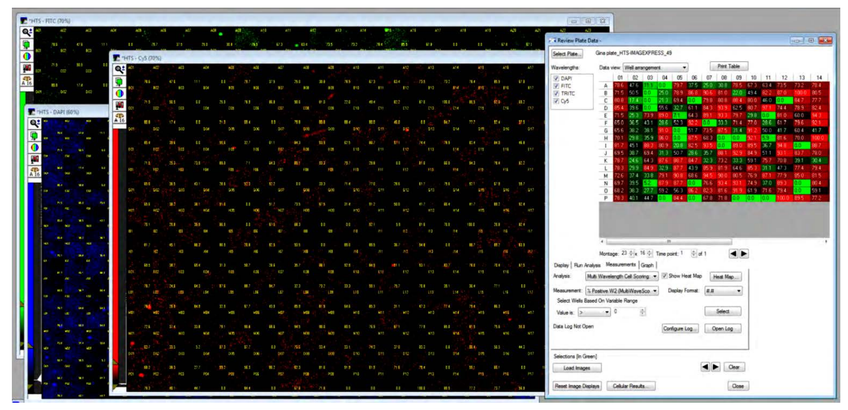


Figure 3: Heatmap shows results of drug treatment and siRNA knockdown of cells.

By using HCS, multiparametric cytotoxicity assay that simultaneously measures (a) nuclear morphology (size, aspect ratio, and texture), (b) mitochondrial function (mass, transmembrane potential, and distribution), (c) plasma membrane permeability, and (d) cell proliferation can be determined.

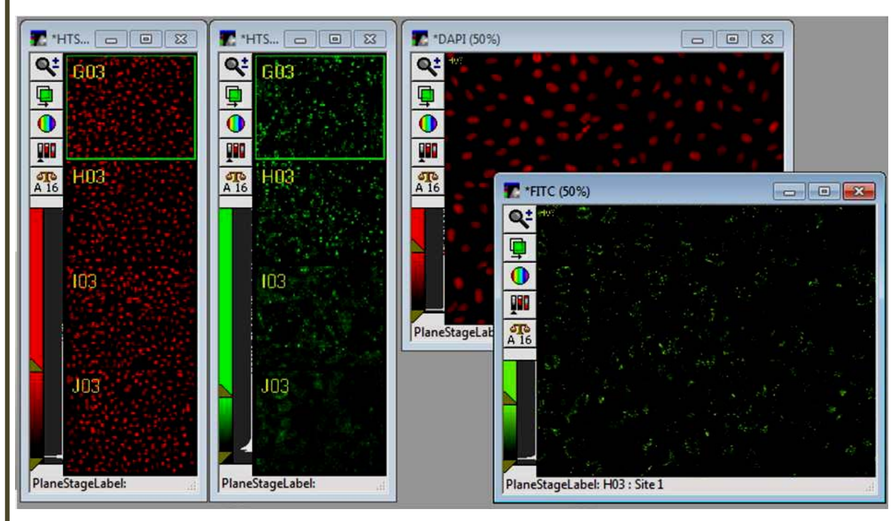


Figure 4: Image of cells being stained with Hoechst 33342 (blue) and Propidium Iodide (red).

CONCLUSION

The ImageXpress micro widefield HCS system is a fully integrated hardware and software system for automated acquisition and analysis of images for high-throughput cell-based cytotoxicity and other testing. When configured with the optional environmental control, it can monitor living cell responses or kinetic reactions in real-time for up to several days. Saved images can be reviewed any time and analyzed with one of the MetaXpress® software.

References:

1. Abraham, V. C., Towne, D. L., Waring, J. F., Warrior, U., & Burns, D. J. (2008). Application of a high-content multiparameter cytotoxicity assay to prioritize compounds based on toxicity potential in humans. Journal of biomolecular screening.
2. Giuliano, K. A., Haskins, J. R., & Taylor, D. L. (2003). Advances in high content screening for drug discovery. *Assay and drug development technologies, 1*(4), 565-577.