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A Laser Based High Content Analysis Platform Improves Speed, Sensitivity and Axial Resolution for Quantitative Imaging

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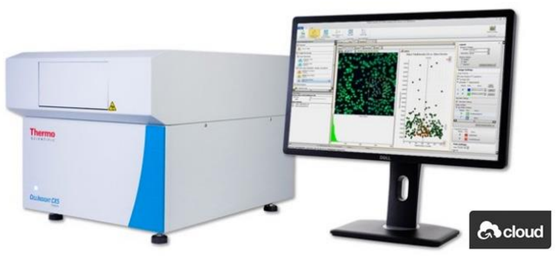
ABSTRACT

High content analysis (HCA) is an automated imaging approach widely used for phenotypic screening in biological research and drug discovery, providing precise quantitation and spatial resolution of fluorescent or colorimetric signals from live and fixed biological specimens. Owing to needs for greater physiological relevance and modeling of living systems, the sample landscape has changed in recent years, shifting from traditional monolayer cultures to include increasingly complex preparations including 3D spheroids, patient derived stem cell lines and organoid cultures. While traditional HCA instrumentation implements wide field imaging with solid state LED or lamp based excitation for maximum throughput and analysis of traditional 2D cultures, such platforms lack the sensitivity and axial resolution needed to get meaningful data from 3D cultures. Here, we made a series of measurements from 2D and spheroid preparations, comparing confocal and wide field laser-based (LZR) excitation on the CellInsight CX7 HCA platform. Looking at measurements of cytoskeletal rearrangement, neurite outgrowth and cell survival in spheroid cultures, we found that confocal laser excitation gave improved axial resolution and penetrance of excitation light when making confocal measurements. Combining these capabilities with a functional on-stage incubator and intuitive software, we found these upgrades to the CX7 light engine greatly improved scan rates, spatial resolution and performance when collecting data from complex samples like 3D spheroids, primary neurons, and cytoskeletal rearrangement assays.

INTRODUCTION

CX7 LZR is a high content imaging instrument that now comes with laser excitation that reduces scanning times and improves axial resolution in 2D and 3D cultures. CX5 operates in five channels with LED excitation while CX7 LZR is a seven laser system with 405, 458, 488, 561, 594, 647 and 785 nm.

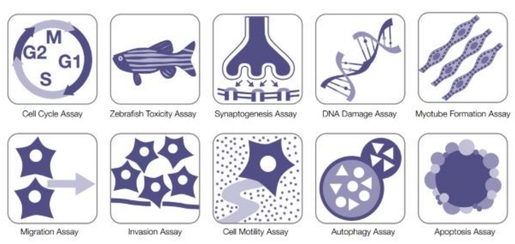


CellInsight CX7 LZR system highlights

Illumination	7-channel, laser-based illumination
Camera	Photometrics X1 camera
Widefield	7-channel fluorescent imaging
Brightfield	5-channel brightfield imaging
Confocal	7-channel confocal imaging
Objectives	3-position objective (range from 2x–40x, low and high NA)
Focus	Software and laser-based autofocus for consistent scan times
Automation	Configured for fully automated plate handling and scanning
Software	HCS Studio software for integrated data collection and analysis

Even more advanced performance through powerful laser illumination light source

The Thermo Scientific CellInsight CX7 LZR High Content Analysis (HCA) Platform is a fast, laser-based, automated cellular imaging and analysis platform for quantitative microscopy and phenotypic screening. It's designed to provide you with the **sensitivity and speed** that's needed for emerging assays.



RESULTS

Monitoring Neural Survival and Growth

Figure 1. Quantitative imaging of survival in B27™ and B27™ Plus medium

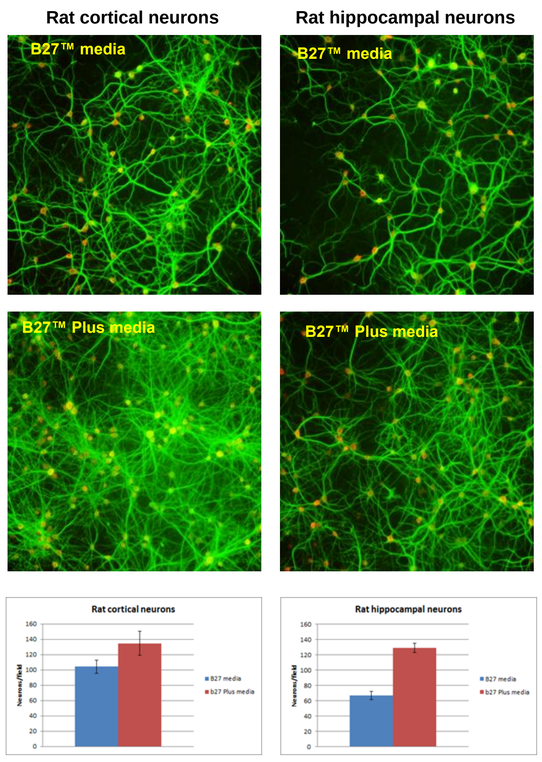


Figure 1. B-27™ Plus Neuronal Culture System enables better survival of primary rodent neurons compared to B-27™ Supplement and Neurobasal Medium. Cryopreserved neurons were cultured for 3–4 weeks in the indicated media system, using first-generation B27™ supplement and Neurobasal™ media or B-27™ Plus supplement with Neurobasal™ Plus culture medium. At the time of analysis, neurons were fixed and immunostained for the neuronal dendritic marker MAP2 (green) and the neuronal cell body marker HuC/D (red). The scanning and quantitation was done on a CellInsight CX7 LZR HCA instrument.

Figure 2. Monitoring neuronal toxicity with CX7 LZR HCA system

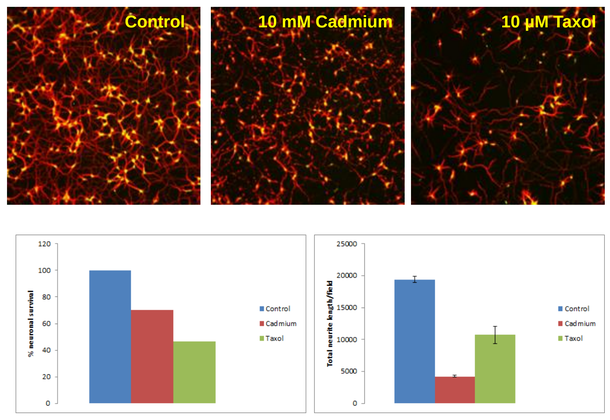


Figure 2. Quantitative assessment of neuronal toxicity. Rat cortical neurons were cultured for 2 days in a 96-well plate and treated with 10 mM cadmium chloride or 10 µM taxol for 24 hrs. Cells were then stained with neuronal dendritic marker,MAP-2 (red) and neuronal cell body marker, HuC/D (green). The scanning and quantitation was done in confocal mode using the CellInsight CX7 LZR HCA instrument.

Imaging Spheroid Cultures with CX7 LZR

Figure 3. Automated scanning of spheroids in bright field

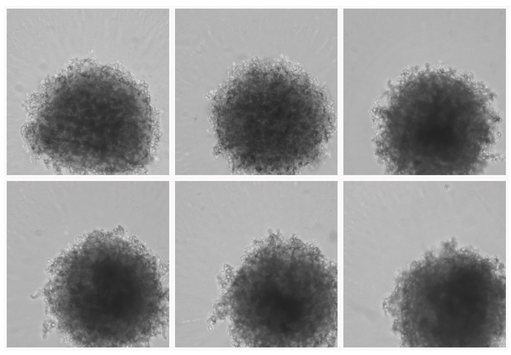


Figure 3. CX7 LZR system allows for automated imaging of spheroids. A549 cells were plated at a density of 5K/well on a corning U-bottom plate and incubated for 48 hrs in the CO2 incubator. The plate was automatically imaged with 10X objective using brightfield illumination on a CellInsight CX7 LZR HCA instrument.

RESULTS CONT.

Figure 4. Improved axial resolution in confocal mode

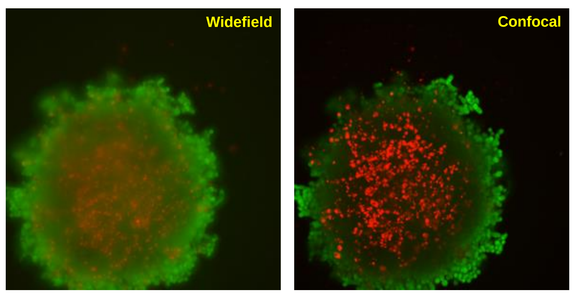


Figure 4. Confocal imaging on CX7 LZR HCA system improves axial resolution of 3D spheroid imaging. A549 cells were plated at a density of 5K/well on a corning U-bottom plate and incubated for 48 hrs in the CO2 incubator. The plate was automatically imaged with 10X objective using wide field or confocal modes with both 561 and 488 lasers on a CellInsight CX7 LZR HCA instrument. The image is from a maximum intensity projection of 20 optical Z slices of 10 microns each.

Figure 5. Automated confocal imaging of spheroids

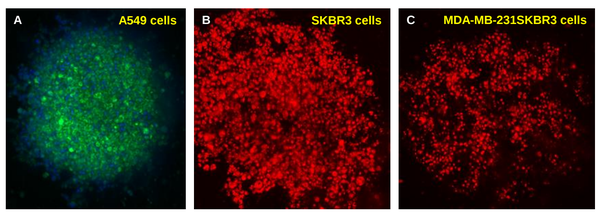


Figure 5. Confocal imaging on CX7 LZR HCA system allows measurement of hypoxic conditions and receptor internalization
Figure 5a: A549 cells were plated at a density of 5k cells/well in a 96-well Corning U-bottom plate and incubated for 48 hrs under normoxic conditions. The spheroids were then stained with 5 µM Image-iT™ Hypoxia Green probe (green) and Hoechst 33342 (blue). The plate was automatically imaged with 10X objective in confocal mode on a CellInsight CX7 LZR HCA instrument. The image is from a maximum intensity projection of 20 optical Z slices of 10 microns each.
Figure 5b and 5c: HER2+ SKBR3 or a combination of SKBR3 and HER2 – MDA-MB-231 cells (50:50) were plated in Corning U-bottom plates at a density of 10k cells/well and incubated o/n in a CO2 incubator. The spheroids were then incubated with Herceptin-pHrodo red conjugates for 48 hrs at a concentration of 1µg/ml to probe relative abundance of internalized HER2 receptors. The plate was automatically imaged with 10X objective using confocal mode on a CellInsight CX7 LZR HCA instrument using the available onstage incubator system. The image is from a maximum intensity projection of 20 optical Z slices of 10 microns each.

Figure 6: Monitoring oxidative stress in spheroids

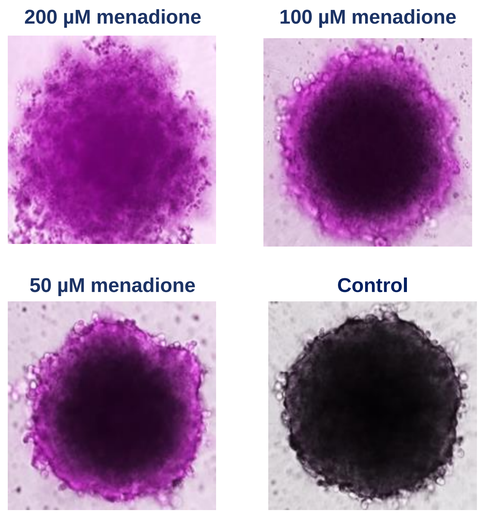


Figure 6. Endpoint measurement of ROS in spheroids. A549 cells were plated at a density of 5k cells/well in a 96-well Corning U-bottom plate and incubated for 48 hrs under normoxic conditions. The spheroids were then stained with 5 µM CellROX™ Deep Red (red). The plate was automatically imaged with 10X objective using confocal modes on a CellInsight CX7 LZR HCA instrument. The image is from a maximum intensity projection of 20 optical Z slices of 10 microns each and overlaid onto a brightfield image for display.

RESULTS CONT.

Near-IR Multiplex Imaging of Cytoskeleton

Figure 7. Quantitative imaging of actin disruption

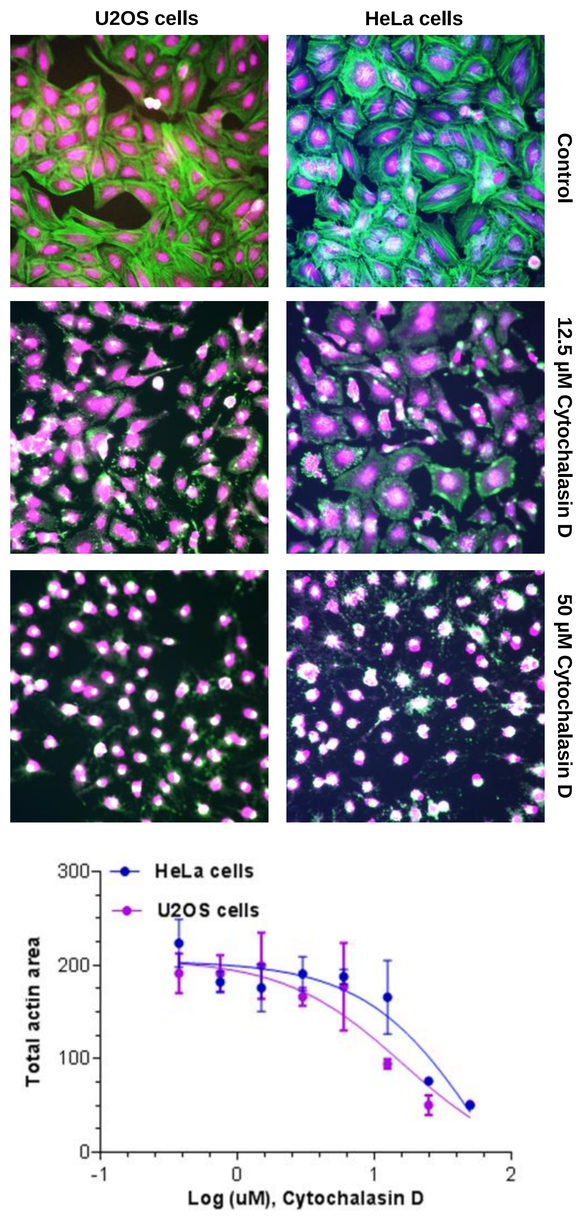


Figure 7: Cytochalasin D disrupts actin filaments and reduces the total area of actin in cells. HeLa or U2OS cells were plated on a 96-well plate at a density of 5k cells/well. The cells were treated with different doses of cytochalasin D with the highest concentration at 50 µM and lowest at 0.375 µM for 4 hrs. The cells were then fixed and permeabilized. The cells were stained with tubulin antibody (AF594 secondary) and AF488 phalloidin. The cells were then washed and stained with HCS Cell Mask 800 and Hoechst 33342. The cells were imaged and analyzed on a CellInsight CX7 LZR HCA instrument using 20x objective. The mean fiber area (actin) was plotted against the cytochalasin D dose.

CONCLUSIONS

New needs in quantitative imaging and High Content Analysis call for imaging capabilities in standard 2D models and a variety of 3D model systems, often using live cell cultures in controlled atmospheric and temperature conditions. Here, we demonstrate amenability of the CX7 LZR laser excitation system to the capture and quantification of imaging data in widefield, confocal and brightfield illumination conditions across a variety of relevant model systems, including primary neural cultures, oncogenic spheroid and standard 2D culture systems. There is also an available Onstage Incubator (OSI) module to enable chronic, dynamic measurements in live cell conditions. Live cell examples here include the use of Image-IT™ hypoxia sensor and pH sensitive pHrodo™ indicator dye systems. In addition to these capabilities, we captured and quantified signals from standard 2D systems, looking at neural survival, neurite outgrowth, ROS production and cytoskeletal rearrangements with new-generation long wave/near IR dye tools compatible with the onboard 785 nm laser excitation. We saw dramatic improvements in axial resolution in 3D spheroid cultures, enabling ready quantification of signals from the surface and internal conditions with greater precision and quality than standard widefield excitation.

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