High-throughput confocal imaging of spheroids for screening cancer therapeutics

Introduction
In recent years, there has been significant progress in development of in vitro aggregates of tumor cells for use as models for in vivo tissue environments. When seeded into a well of a low-attachment round bottom microplate, these aggregates will form a discrete spheroid. Spheroids are believed to mimic tumor behavior more effectively than regular two dimensional (2D) cell cultures because, much like tumors, they contain both surface-exposed and deeply buried cells, proliferating and non-proliferating cells, and a hypoxic center with a well-oxygenated outer layer of cells. Such 3D spheroid models are being successfully used in screening environments for identifying potential cancer therapeutics.

Some challenges to developing robust spheroid assays:
• Locating and focusing on the spheroid in every well so it can be imaged in a single field-of-view
• Optimizing the compound and staining treatment to ensure dye penetration and avoid disturbing the spheroid placement
• Acquiring representative images throughout the 3D structure, minimizing out-of-focus or background signal from above and below the imaging plane

APPLICATION NOTE
• Rapidly analyzing the images to yield meaningful results from which conclusions can be drawn.

Spheroid formation and treatment
We used the following method to form spheroids from cancer cell lines HCT116, DU145, and HepG2. Cells were cultured in flasks at 37 °C and 5% CO₂ before detaching and seeding into 96 or 384-well black plates with clear bottom U-shaped wells (Corning 4520 and 3830, respectively) at densities of 1000-1500 cells/well in the appropriate media supplemented with fetal bovine serum (FBS). Within 24 hours, a single spheroid formed in the bottom of each well and continued growing in size until it was used for experimentation after 2-4 days at 37 °C and 5% CO₂. Spheroids may be cultured longer but the increasing size may impede stain penetration and imaging of the center-most cells. This application note describes assays used to determine the effects of the anti-cancer compounds: etoposide, paclitaxel, and Mitomycin C. Spheroid treatment began by adding compounds into the wells at

Key features
• Acquire statistically relevant data quickly with an advanced scientific CMOS detector, enabling >3 log dynamic range
• Improve visualization and quantitation with 3D assay models
• Achieve excellent image quality without sacrificing throughput using our unique optical path technology
• Expand your research capabilities with water immersion objectives, transmitted light, phase contrast optics, on-board liquid handling, and environmental control options

For researchers looking to expand their laboratory's capabilities, the ImageXpress Micro Confocal system leverages large field-of-view optics to map macrostructures with minimal tiling. In addition, querying of large cell populations is accelerated, speeding up the characterization of highly heterogeneous samples or identification of rare subpopulations.
Specifications

System

• High-speed laser autofocus with integrated image autofocus option
• Linear encoded voice coil driven X, Y, and Z stages with < 25 nm resolution
• 4-position automated objective changer*
• 5-position software selectable dichroic filter wheel*
• 8-position software selectable emission filter wheel*
• Sample compatibility: slides and one to 1536-well microplates, round or flat bottom, low to high profile, and Transwell® plates

AgileOptix optical path

• AgileOptix™ technology enables the ImageXpress Micro Confocal system to deliver the sensitivity and throughput needed for demanding applications by combining a powerful solid-state light engine, high-quantum efficiency 16-bit, >4 megapixel scientific CMOS sensor, and selectable unique confocal geometries
• >3 log dynamic range is available in both widefield and confocal modes
• Large field of view (1.96 mm² at 10X) imaging maximizes collection of publication quality images and statistically relevant data

*User changeable

Option Feature

Water Immersion Objectives
• 20X, 40X, and 60X (up to 1.2 NA)
• Increase signal up to 4 times for brighter intensity at lower exposure times
• Increase in penetration depths dependent on sample
• Improve z-resolution and decrease optical aberrations
• Auto water replenishment enables screening or imaging across a plate

Environmental Control
• Multi-day, live cell time-lapse imaging
• Provides appropriate atmospheric conditions (e.g. 5% or 10% CO2)
• Mimics physiological environment (30–40 °C ± 0.5 °C)
• Controls humidity and minimizes evaporation (0.5 µL/well/hour for 96- or 384-well formats)

Phase Contrast
• High contrast imaging where unstained cells are easily viewed or separated from background (4X–60X)
• Ideal for non-fluorescent histochemically stained samples
• Nikon 100W Pillar Diascopic Illuminator with TE-C ELWD Condenser
• 0.3 NA with 65 mm WD and PhL, Ph1, and Ph2 selectable phase rings
• Fluorophore-independent morphology visualization with fluorescent imaging overlay

On-board Fluidics
• Single-channel pipettor
• Dispense volumes from 3 µL to 200 µL (±1 µL; ±5%)
• Compatible with 96- or 384-well format FLIPR System pipette tips
• Holds two plates for compound addition or media exchange
• Optional plate heating
• Environmental control

Note: all options, filters, and objectives are available at point of sale or as after market upgrades. Configurations shown herein do not encompass all configurations available. Contact your sales and support team today to identify the system configuration most suitable for your applications.

Implement a solution that works for you

Molecular Devices can successfully tailor the ImageXpress Micro Confocal High-Content Imaging System to include customized software and hardware including the features described herein, as well as integration of other lab components such as incubators, liquid handlers, and robotics for a fully automated workcell. With over 30 years of experience in the life science industry, you can count on us to deliver quality products and provide worldwide support.

Sale is subject to our Custom Product Purchase Terms available at www.moleculardevices.com/custom-products-purchase-terms.