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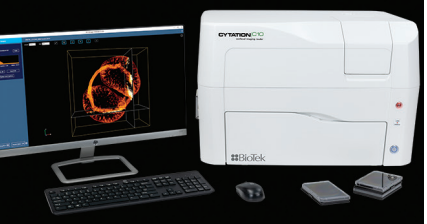
Application Bulletin

Confocal Imaging and Analysis of Spheroids for Determination of Dose Response during Drug Treatment



Key Words:

3D
Spheroids
HCT116 Cells
Tumoroids
Confocal Imaging
Z-stack
Fluorescent Imaging

Introduction

The last decade has seen an explosion in the use of cells and tissues from a wide range of sources cultured in a three-dimensional (3D) setting resulting in more complex biological models. The application of these 3D models has been on the increase in areas such as medical research, precision medicine, disease modeling and drug discovery efforts. These models tend to be representative of the native microenvironments found in organisms and are thought to provide a more accurate assay model in some instances. 3D models are often compared to the widely used two-dimensional (2D) models, those consisting of a monolayer of cells, which have been in use for decades. The use of 2D assays has provided meaningful data but limitations became apparent and more recently the development of complex organoids, tissues or tumoroids comprised of one or more cell types, often based on stem cells or patient derived samples, have become the focus of many studies.

The ability to perform a variety of assay types on a single instrument with a small footprint can be advantageous given the limited bench space found in many laboratories. The Cytation C10 confocal imaging reader performs microplate reading, widefield imaging as well as confocal imaging on a single instrument, providing a means to gather the wide range of data needed for many of today's complex studies. This application bulletin describes the use of an automated imaging assay for accurate determination of cell number in spheroids for evaluation of drug dose response.

Materials and Methods

HCT116-H2B-GFP cells were provided by the Stumpff lab at the University of Vermont. The H2B-GFP construct provided a constitutively expressing nuclear marker for quantification by cell count for comparison to a nuclear stain during live-cell analysis. The cells were seeded at a relatively low density of 500 cells/well in a 96-well, round bottom ULA imaging plate, part number 4520, from Corning (Corning, NY) and allowed to grow for 3 to 4 days, depending on conditions. This resulted in spheroids of roughly 100 µm in size. The potent, non-selective inhibitor of protein kinases, staurosporin, was added as a known inducer of apoptosis. Propidium iodide (PI) was added simultaneously to monitor the events kinetically for determination of optimum analysis timing. It was determine that 12 hours post-addition was sufficient to show significant apoptotic activity for analysis.

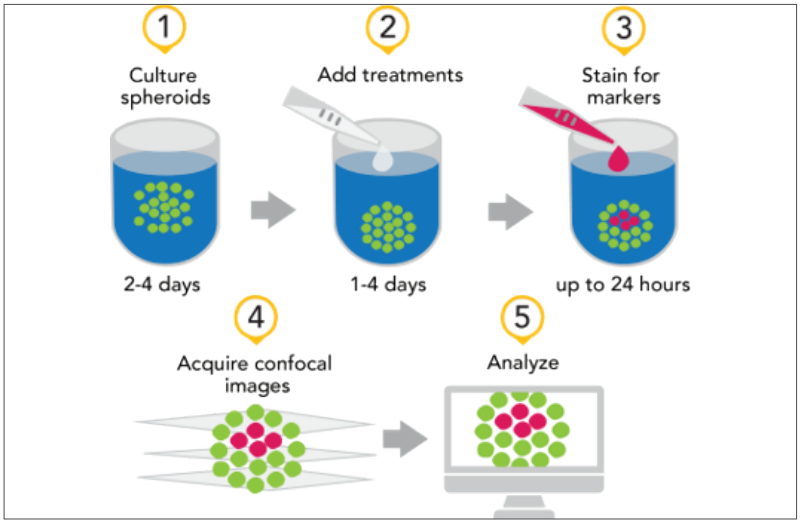


Figure 1. Spheroid assay workflow incorporating automated image acquisition and analysis conducted with the Cytation C10 confocal imaging reader and Gen5 microplate reader and imager software.

BioTek Instruments, Inc.
P.O. Box 998, Highland Park,
Winooski, Vermont 05404-0998 USA
Phone: 888-451-5171
Outside the USA: 802-655-4740
Email: **customercare@biotek.com**
www.biotek.com

Imaging Procedure and Analysis

The sizing of the spheroids was deliberate allowing the entire object to be captured, in a single field of view when using a 20x objective, to minimize the size of the data set. Additionally, a beacon was used to account for any variability in x-y positioning of the spheroid within the wells to keep to a single field of view. The 60 µm pinhole spinning disk was found to provide the best compromise between acquisition time and signal intensity for confocal imaging. Widefield images were captured in both GFP and TRITC channels for comparison to confocal images.

The focus method used was "Scan", followed by "Autofocus". This focus method identifies the approximate center of the spheroid along the z axis and allows each spheroid to be captured as a z-stack using 11 steps at 10 µm regardless of the z position in the microplate well. The images were subject to several processing steps including a z-projection for comparison of a single z-plane image at the approximate center of the spheroid and the entire z-stack for both imaging modes.

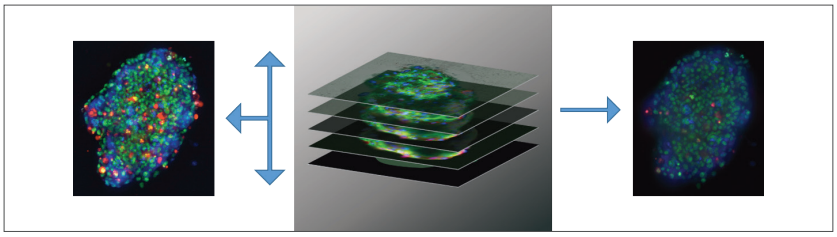


Figure 2. The spheroid was captured as a z-stack using 11 steps at 10 µm intervals. Analysis was performed on either a z-projection of all the images or a single image representing a plane through the approximate center of the spheroid.

Automated cell counting was performed using confocal and widefield imaging modes, with analysis conducted on both single plane and z-stack projected images.

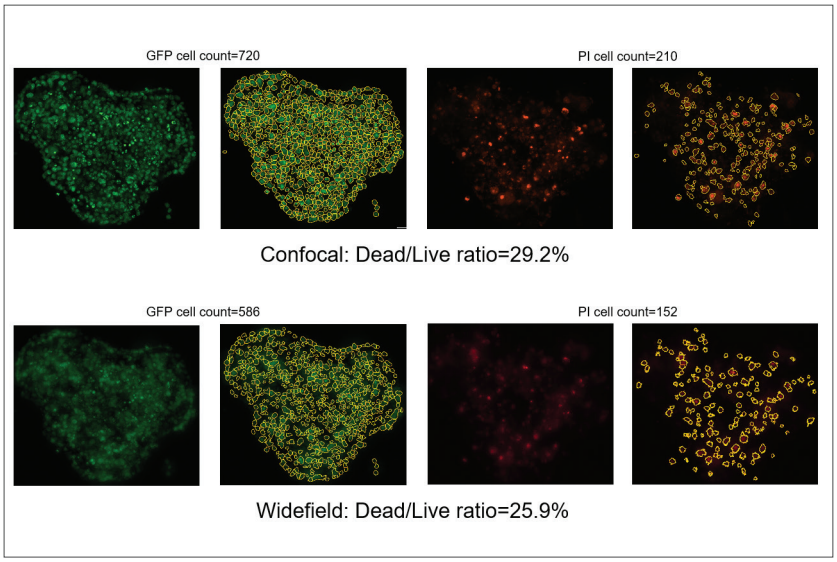
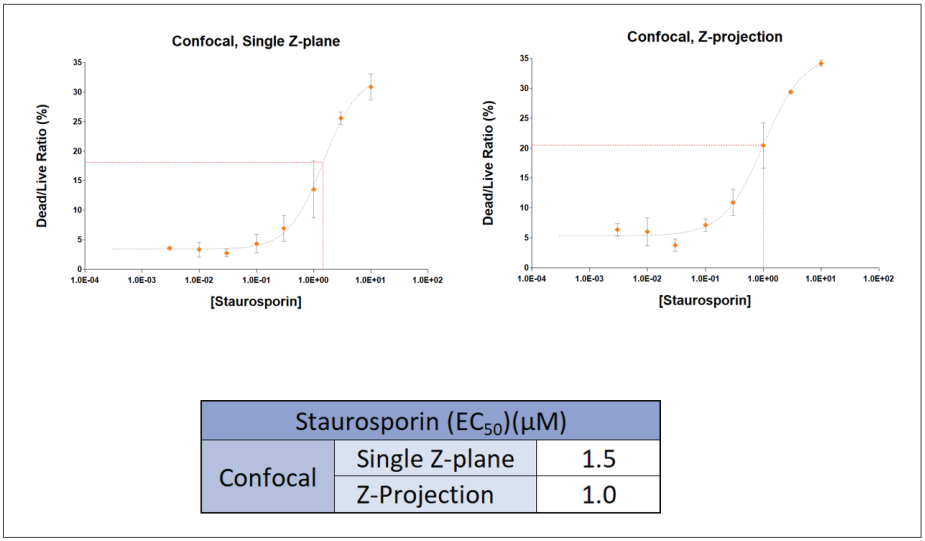


Figure 3. Representative examples of automated image acquisition and cell count analysis of spheroids by confocal and widefield fluorescent microscopy using z-projected images. Cell masking represented by yellow outlines where GFP cell count indicates live cells and PI indicates dead cells.



Staurosporin (EC_{50})(µM)		
Confocal	Single Z-plane	1.5
	Z-Projection	1.0

Figure 4. EC_{50} determinations for each imaging mode, single versus z-projected images.

Conclusion

Spheroids are increasingly used to represent the more complex microenvironments indicative of biological systems. The ability to automate image capture using multiple imaging modes and perform automated image analysis can significantly increase throughput and allow for comparative analysis. Visual inspection of results confirmed confocal optics enable more accurate segmentation of labeled cells within spheroid samples compared to widefield optics. Cell count determination using a single z-plane image was found to provide comparable results to methods relying on z-stack image acquisition and processing. This study provides an example of the flexibility of the Cytation C10 confocal imaging reader and Gen5 microplate reader and imager software to capture and analyze multiple parallel data sets, using a single, compact instrument, to help simplify assay development and optimization.

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