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Cell imaging

Automated fluorescence imaging and quantification of cell viability

Introduction

Measuring cell viability is a key tool for studying cytotoxicity of compounds, infection, disease, and other aspects of cell health. While there are many cell viability and toxicity assays for high-throughput analysis, quantifying viability based on imaging of live and dead cells has typically been difficult and time-consuming. This makes it challenging to analyze a large number of samples and obtain quantitative data. With the recent introduction of automated fluorescence imaging systems, optimized viability staining kits, and powerful image analysis software, it is now possible to quickly and easily generate high-quality fluorescence images for many samples and quantify the percentage of live and dead cells to obtain cell viability data with high statistical power.

The Invitrogen™ LIVE/DEAD™ Cell Imaging Kit (488/570) is a sensitive assay that uses two-color fluorescence to determine cell viability. Quick and easy to use, the kit allows discrimination between live and dead cells using two probes that measure recognized and different parameters of cytotoxicity and cell viability. Intracellular esterase activity is determined using a cell-permeant dye to stain live cells. Plasma membrane integrity is determined using a cell-impermeant dye to stain dead and dying cells with compromised cell membranes. To adapt this assay for imaging platforms, the LIVE/DEAD Cell Imaging Kit components were optimized for the common green and red imaging filters used for GFP and Texas Red™ dye fluorescence detection. The live-cell detection component calcein AM produces an intense, uniform green fluorescence (ex/em: 488 nm/515 nm) in live cells with esterase activity, while the dead-cell detection component produces nuclear red fluorescence (ex/em: 570 nm/602 nm) in cells with compromised cell membranes (Figure 1). The live- and dead-cell detection components of the kit are configured for ease of use with minimal dilutions, so the staining can be performed quickly on many samples.

The Invitrogen™ EVOS™ M7000 Imaging System enables high-quality, automated fluorescence imaging of cells grown and stained on or in a variety of vessels, from coverslips to entire 96-well plates. The EVOS M7000 system, therefore, allows large numbers of cells labeled with the two-color LIVE/DEAD Cell Imaging Kit to be imaged quickly and easily. The fluorescence images can then be analyzed and quantified using Invitrogen™ Celleste™ Image Analysis Software to obtain the percentage of live and dead cells after cells are exposed to various treatments and conditions. The optimized reagents, automated imaging system, and robust analysis software can be used together to perform a simple protocol to obtain cell viability data from cells treated with varying concentrations of gambogic acid, a toxic compound known to affect cell and tumor growth.

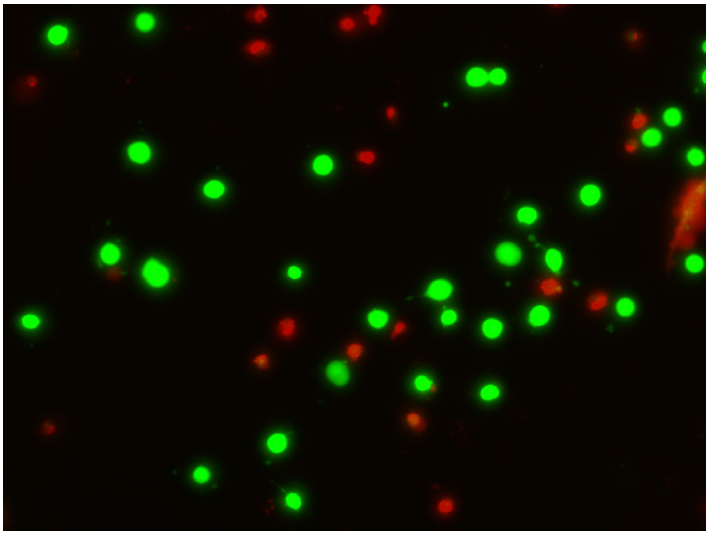

Figure 1. A549 cells treated with gambogic acid overnight and stained with the LIVE/DEAD Cell Imaging Kit. Cells were imaged using GFP and Texas Red light cubes on an EVOS M7000 Imaging System. Live cells with intracellular esterase activity have green fluorescence, and dead cells with compromised membranes have red fluorescence.

Materials

- A549 cells
- Gambogic acid
- LIVE/DEAD Cell Imaging Kit (Cat. No. R37601)
- Invitrogen™ NucBlue™ Live ReadyProbes™ Reagent (Cat. No. R37605)
- EVOS M7000 Imaging System (Cat. No. AMF7000)
- Celleste Image Analysis Software (Cat. No. AMEP4942)
- Invitrogen™ EVOS™ Light Cube, GFP 2.0 (Cat. No. AMEP4951)
- Invitrogen™ EVOS™ Light Cube, Texas Red 2.0 (Cat. No. AMEP4955)

Methods

A549 cells were plated in a 96-well plate at 5,000 cells/well. Cells were treated overnight with gambogic acid at concentrations ranging from 0 to 40 µM, with four wells per treatment condition for a total of 48 wells. The next day, cells were labeled with the live and dead detection components of the LIVE/DEAD imaging kit. After thawing the vials, the contents of one Live Green vial (component A), containing 1 mL of a 1 µM solution, were transferred to a vial of the lyophilized Dead Red reagent (component B). The solution was mixed well to obtain a 2X working solution and added to an equal volume of the cells in medium. The NucBlue Live reagent was added at two drops per mL of medium, and the cells were incubated in the LIVE/DEAD staining solution for 15 minutes at 25°C, followed by imaging on the EVOS M7000 system. After setting the magnification, focus, and channel illumination parameters, an automated image acquisition routine protocol was set to scan 48 wells by autofocusing on the signal of the NucBlue Live reagent. Four fields per well were scanned and imaged (Figure 2). A total of 576 images were obtained, overlaid to generate 192 fields in three fluorescence colors. The total scan time was only 13 minutes. The LIVE/DEAD analysis protocol from Celleste 6 Image Analysis Software was used to analyze the images (Figure 4). Segmentation parameters were set using the blue-fluorescence to define total cells, green-fluorescence to define live cells and red-fluorescence to define the dead cells. Running the protocol generated the total, live and dead cell counts, which were displayed in the data table along with population calculations and subsequently exported to a Microsoft™ Excel™ file. The percentages of positive cells for the live green and dead red staining was plotted against the gambogic acid concentration.

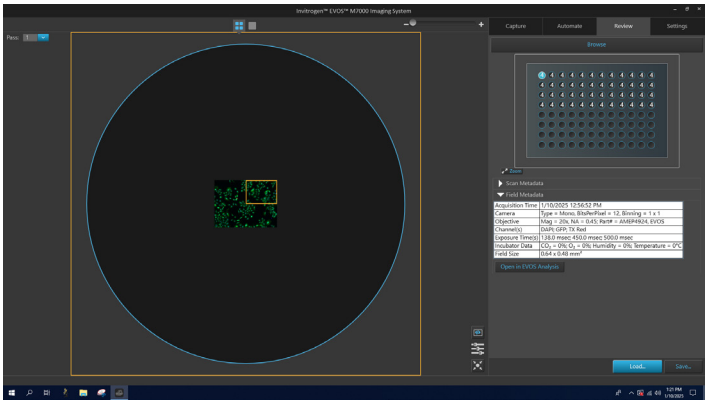

Figure 2. The automated acquisition routine for cells stained with the LIVE/DEAD Cell Imaging Kit. After setting the magnification, focus, and channel illumination parameters, an automated image acquisition routine was set to scan 48 wells by autofocusing on the NucBlue Live signal. Four fields per well were scanned and imaged using the GFP and Texas Red channels. A total of 576 images were obtained in a total scan time of 13 minutes.

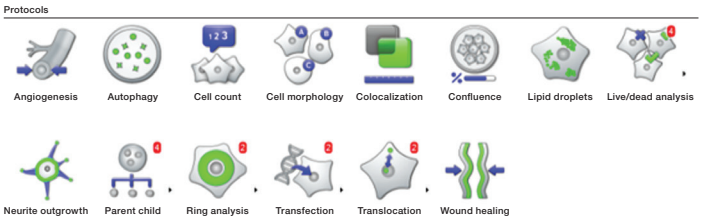

Figure 3. LIVE/DEAD analysis, one of the available Celleste 6 pre-configured protocol templates, was used for batch analysis. The Celleste 6 Multi Channel Analysis (MCA) tool is based on pre-configured algorithms and analysis templates trained on representative data to optimally segment and classify images from a range of common cell-based assays including cell viability, apoptosis, cell cycle, LIVE/DEAD cell analysis, transfection efficiency, immunohistochemistry analysis, migration tracking, wound healing, adipogenesis, and cytoskeletal disruption.

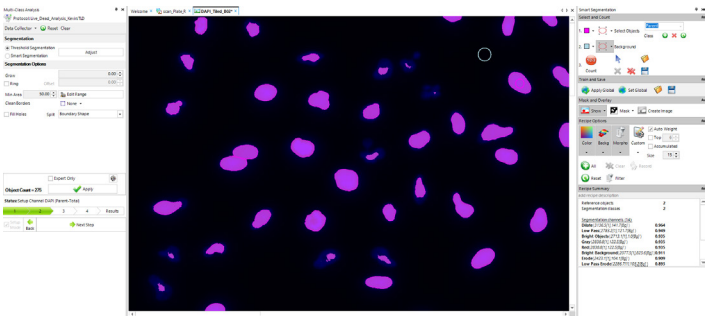

Figure 4. Defining object classes using the smart segmentation tool in LIVE/DEAD protocol. In the LIVE/DEAD protocol, smart segmentation or intensity-based segmentation tools are used to define total, live and dead cells based on staining. DAPI-stained cells were identified and counted as total cell while green-fluorescence cells were defined as live and red-fluorescence cells were defined as dead. After setting segmentation parameters, cells from each image scan were identified and counted with the LIVE/DEAD cell analysis protocol. Total, live and dead cell counts from each scan were then displayed in a data table along with calculated populations.

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Results

In the absence of gambogic acid, nearly 100% of the cells stained positive for Live Green reagent, while increasing the concentration of gambogic acid from 0 to 40 µM led to nearly 100% of the cells staining positive for Dead Red reagent (Figure 5). The EC_{50} for gambogic acid was 4.9 µM with an R_2 of 0.981. Over 12,000 objects in 192 images from the 48 wells were analyzed, resulting in quantifiable data with small standard errors of the means (SEMs).

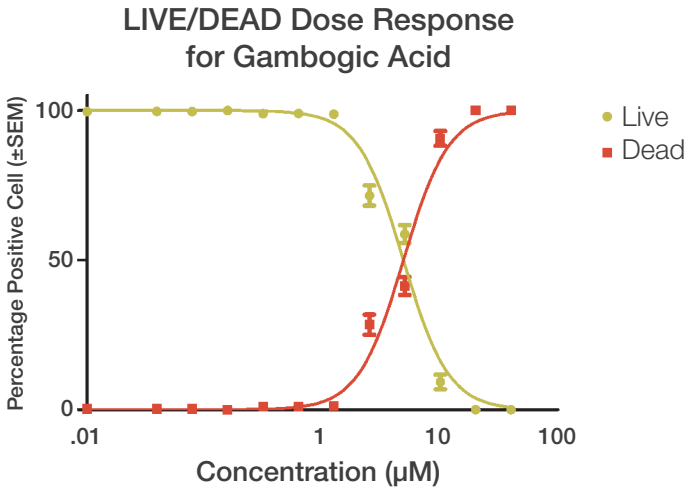

Figure 5. Celleste software analysis of A549 cells treated with gambogic acid and stained with the LIVE/DEAD imaging kit. As the concentration of gambogic acid increased, the percentage of cells positive for Live Green fluorescence decreased and the percentage of cells positive for Dead Red fluorescence increased; The EC_{50} was 4.9 µM with an R_2 of 0.981. Error bars represent SEMs.

Conclusion

Using a simple and optimized procedure for staining live and dead cells with the LIVE/DEAD Cell Imaging Kit, A549 cells treated with gambogic acid were imaged by automated acquisition on the EVOS M7000 Imaging System after defining a few basic parameters. This allowed imaging of cells plated in 48 wells and treated with a range of gambogic acid concentrations, to obtain 192 overlaid images that could be analyzed using an automated object segmentation and counting protocol in Celleste Image Analysis Software. This resulted in both high-quality fluorescence images and quantitative data from a statistically significant number of cells.

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