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 difficult 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™ FL Auto 2 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 FL Auto 2 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.

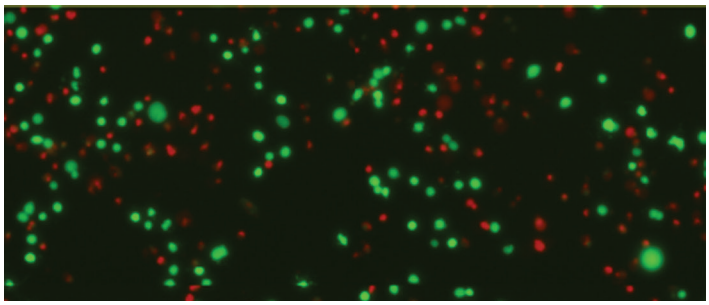

Figure 1. A549 cells treated with gambogic acid overnight and stained with the LIVE/DEAD Cell Imaging Kit. Cells were imaged in the GFP and Texas Red fluorescence channels on the EVOS FL Auto 2 Imaging System. Live cells with intracellular esterase activity have green fluorescence, and dead cells with compromised membranes have red fluorescence.

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Materials

- A549 cells
- Gambogic acid
- LIVE/DEAD Cell Imaging Kit (Cat. No. R37601)
- Invitrogen™ NucBlue™ Live ReadyProbes™ Reagent (Cat. No. R37605)
- EVOS FL Auto 2 Imaging System (Cat. No. AMAFD2000)
- Celleste Image Analysis Software (Cat. No. AMEP4816)

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 4 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 FL Auto 2 system. After setting the magnification, focus, and channel illumination parameters, an automated image acquisition routine 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 12 minutes. Celleste Image Analysis Software was used to analyze the images using the object counting and smart segmentation tools. Green-fluorescent cells were defined as live, red-fluorescent cells were defined as dead, and dark unstained areas were defined as the background (Figure 3). Batch processing was used to allow the segmentation and counting macro to automatically analyze all 192 images for live and dead staining, and the data were 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.

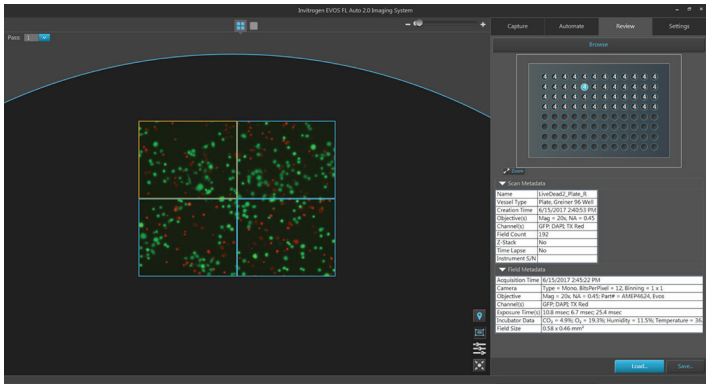

Figure 2. The automated acquisition routine for cells stained with the LIVE/DEAD Cell Imaging Kit. After setting the magnification, focus, and channel illumination parameters, an automated image acquisition routine was set to scan 48 wells by autofocusing on the NucBlue Live signal. Four fields per well were scanned and imaged using the GFP and Texas Red channels. A total of 576 images were obtained in a total scan time of 12 minutes.

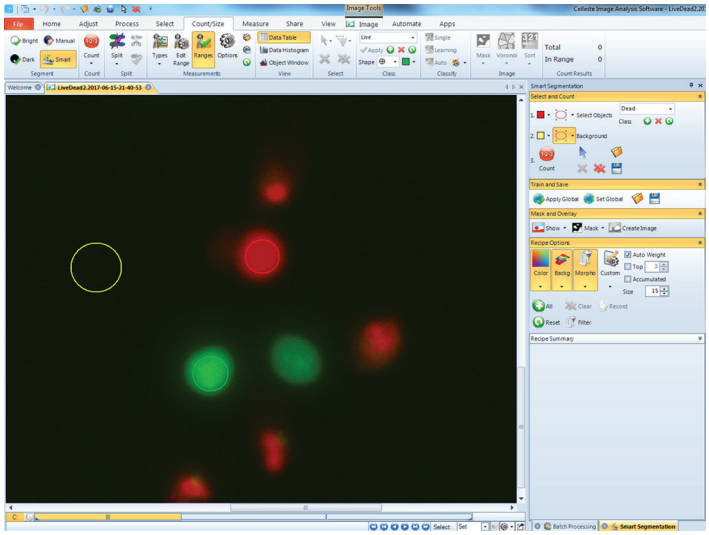

Figure 3. Defining object classes using the smart segmentation tool in Celleste Image Analysis Software. Green-fluorescent cells were defined as live, red-fluorescent cells were defined as dead, and dark unstained areas were defined as the background. Using this object segmentation protocol, the software automatically assigned objects to distinct classes of live and dead cells based on color. This protocol was subsequently applied to all 192 overlaid images obtained from cells treated with a range of gambogic acid concentrations, to generate total cell counts as well as live- and dead-cell counts.

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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 4). The EC_{50} for gambogic acid was 3.7 µM. Over 20,000 objects in 192 images from the 48 wells were analyzed, resulting in quantifiable data with small standard errors of the means (SEMs).

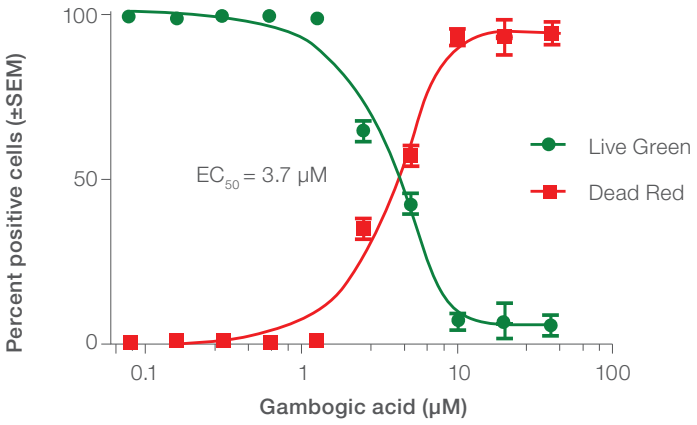

Figure 4. Celleste software analysis of A549 cells treated with gambogic acid and stained with the LIVE/DEAD imaging kit. As the concentration of gambogic acid increased, the percentage of cells positive for Live Green fluorescence decreased and the percentage of cells positive for Dead Red fluorescence increased; EC_{50} was 3.7 µM. 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 FL Auto 2 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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