APPLICATION NOTE

EVOS® FL Auto Cell Imaging System

Collecting Z-stack image sequences on the EVOS® FL Auto Cell Imaging System

Introduction

The EVOS® FL Auto Imaging System is a fully-automated, digital, inverted, multi-channel fluorescence and transmitted-light imaging system with outstanding workflow efficiency. Designed to meet demanding requirements over a broad range of applications, it supports high-resolution mosaic tiling, multi-position well scanning, cell counting with thresholding, and time-lapse studies.

Capturing images at different Z-planes can be a powerful tool in fluorescence microscopy. Depending on the sample type and prep used in the experiment performed, this capability can reveal conditions not seen with standard wide field imaging.

In this application note, a series of images were captured on the EVOS® FL Auto Cell Imaging System. Creating a Z-stack from these images allowed the observation of cellular cytoskeletal changes, which can be indicative of the loss of cell health.

Materials

- CellLight® Tublin-GFP, BacMam 2.0 (C10613)
- CellLight® Mitochondria-RFP, BacMam 2.0 (C10601)
- NucBlue® Live ReadyProbes™ Reagent (R37605)
- EVOS® Light Cube, DAPI (AMEP4650)
- EVOS® Light Cube, GFP (AMEP4651)
- EVOS® Light Cube, RFP (AMEP4652)

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Methods

HeLa cells grown in MatTek 6-well glass bottom culture plates were transduced with CellLights™ Tubulin-GFP and CellLight™ Mitochondria-RFP overnight at 37°C. The following day, NucBlue® Live reagent (2 drops/mL) was added to the cultures. Cells were then imaged on an EVOS® FL Auto Cell Imaging System with 100x oil immersion objective using the Z-stack function. The step size was set using the Nyquist formula and performed at 0.366µm. All images were then processed using ImageJ software.

Results and Discussion

Following transduction and staining of HeLa cells, images were taken using the Z-stacking function on the EVOS® FL Auto Cell Imaging System software. Once the cell of interest was identified, a series of 41 images were automatically captured every 0.366µm of focal plane. Following capture, the 41 images were combined into a movie to allow visualization of the entire cell (Figure 1). Analysis of the composite overlay of step 16 of the Z-stack is indicative of a typical 3-color overlay captured by widefield fluorescent microscopy (Figure 2). In this focal plane, the HeLa cell appears healthy and normal. However, analyzing the movie of the Z-stack series from top to bottom shows the blebbing of tubulin-GFP, which can be an indication of the loss of cell health (Figure 3). These tubulin-GFP blebs are readily apparent in step 26 of the Z-stack image series and not readily apparent in previous Z-stack sections. Using the Z-stack capabilities of the EVOS® FL Auto Cell Imaging System uncovers changes in cellular morphology and possibly cell health not seen at a fixed Z-plane in standard widefield microscopy.

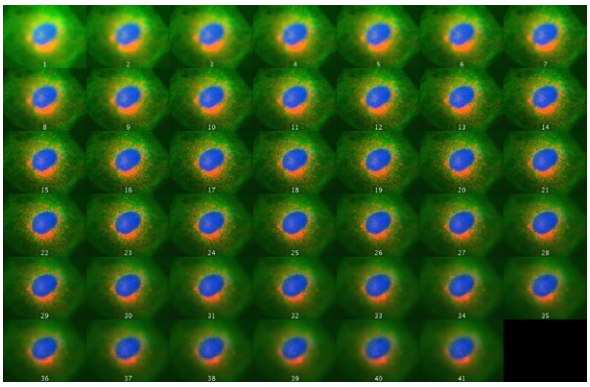

Figure 1. A series of 41 images taken every 0.366µm of focal plane in HeLa cells using the Z-stack function of the EVOS® FL Auto Cell System. These images were combined to create a movie (www.lifetechnolgies.com/evosflautogallery) of the Z-stack sections through the cell.

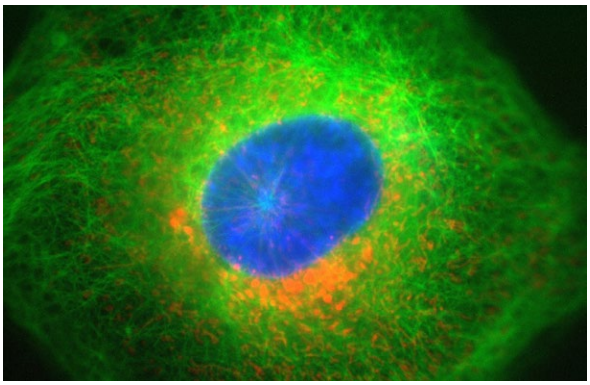

Figure 2. Composite image of step 16 of the Z-stack, indicative of the typical 3-color overlay captured by wide field fluorescence microscopy. Note the intact structure of tubulin molecules (green).

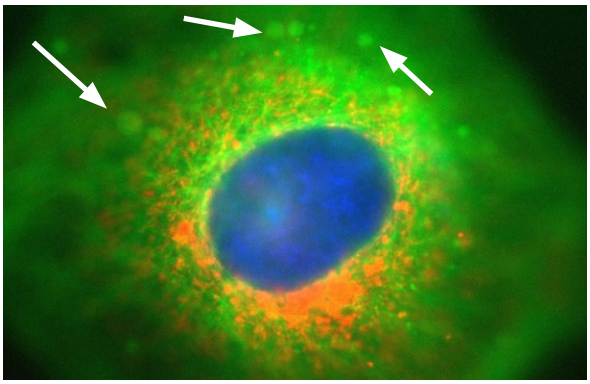

Figure 3. Composite image of step 26 of the Z-stack. Note the blebbing of the tubulin seen in this image, indicative of the loss of cell health.

For more information, visit
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