Quantitation of proliferating cells with the EVOS FL Auto Imaging System

Introduction

The Invitrogen™ 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, multiple-position well scanning, cell counting with thresholding, and time-lapse studies.

Among the versatile software features is cell counting that can be done automatically with a captured or live image. A powerful watershed algorithm has enhanced the precision of counting cells stained with Invitrogen™ Molecular Probes™ NucBlue™ Fixed Cell ReadyProbes™ Reagent. Other nuclear stains/fluorescent proteins, or general cytoplasmic fluorescent dyes/proteins, permit the easy determination of total cell numbers and/or percentage of cells stained for a functional readout.

In this application note, the number of replicating cells was identified as a percentage of the total population of cells. The percentage of proliferating cells was easily determined by labeling cells with EdU-Alexa Fluor™ 594 to identify proliferating cells and NucBlue Fixed Cell ReadyProbes Reagent to identify total cells.

Materials

- Invitrogen™ Molecular Probes™ Image-iT™ Fixation/Permeabilization Kit (Cat. No. R37602)
- Invitrogen™ Molecular Probes™ Click-iT™ EdU Alexa Fluor™ 594 Imaging Kit (Cat. No. C10339)
- NucBlue Fixed Cell ReadyProbes Reagent (Cat. No. R37606)
- Invitrogen™ EVOS™ Light Cube, DAPI (Cat. No. AMEP4650)
- Invitrogen™ EVOS™ Light Cube, Texas Red™ (Cat. No. AMEP4655)

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Methods

HeLa cells grown in a 96-well plate were pulsed with EdU for 1 hour. Cells were fixed, permeabilized and blocked using the Image-iT Fixation/Permeabilization Kit. Following fixation and permeabilization, nuclei were labeled with 2 drops/mL of NucBlue Fixed Cell ReadyProbes Reagent and the incorporated EdU was detected with Alexa Fluor 594 azide (Component B from Cat. No. C10339). Cells with incorporated EdU are shown in pink while all cells have nuclei labeled in blue (Figure 1). Following sample preparation, cells were imaged on the the EVOS FL Auto Imaging System using the EVOS DAPI light cube to visualize NucBlue Fixed Cell ReadyProbes Reagent and the EVOS Texas Red light cube to visualize Click-iT EdU using a 10x objective. Cells were quantitated using the Cell Counting tab on the EVOS FL Auto Imaging System.

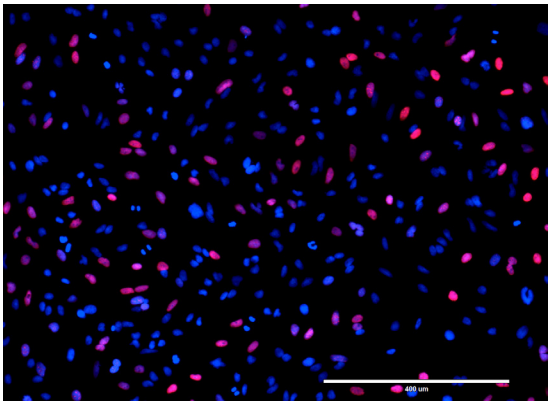

Figure 1. Overlay of 10x image used in the quantitative analysis of proliferating HeLa cells.

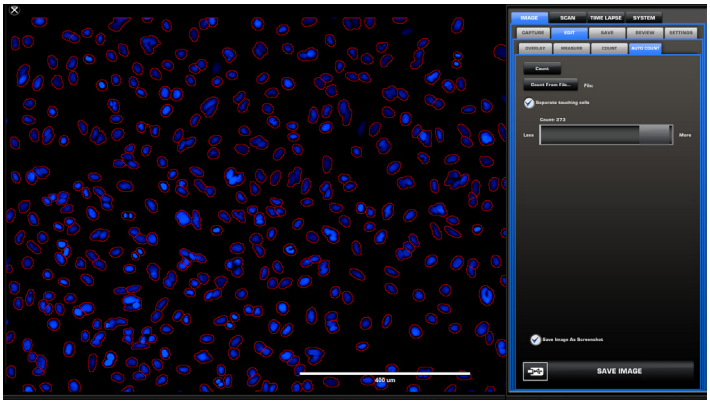

Figure 2. Screen capture of the quantitation of the total population of cells (blue) within the image. 373 total cells were counted in this field of view.

Results and discussion

Total cell population was determined to be 373 by counting the number of cells in the DAPI channel corresponding to NucBlue Fixed staining (Figure 2). Within this population, the number of proliferating cells was determined to be 138 by counting the number of cells in the Texas Red channel corresponding to Click-iT EdU-positive cells. Based on these results, 37% (138/373) of the cells within this population were shown to be actively proliferating (Figure 3). Using the EVOS FL Auto Imaging System allows researchers to combine qualitative images with quantitative measurements to provide rich data for research applications.

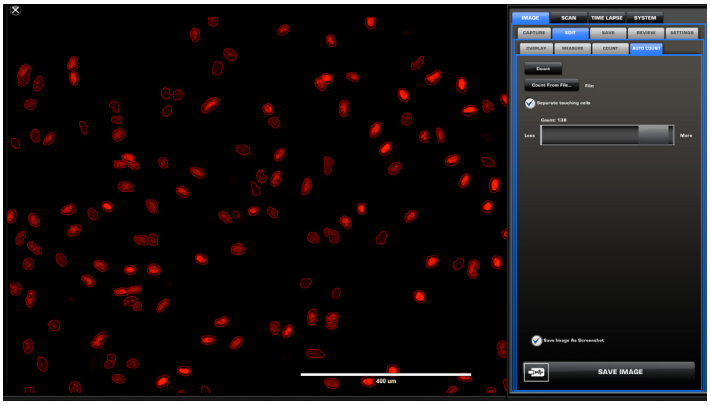

Figure 3. Screen capture of the quantitation of the total population of proliferating cells (red) within the image. 138 proliferating cells were counted in this field of view.

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