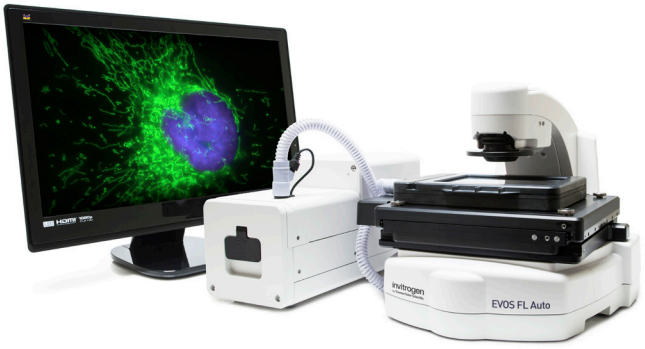
Cell division and migration during wound healing visualized on the EVOS FL Auto Imaging System

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.

Time-lapse imaging is a powerful technique allowing researchers to visualize cellular events and morphology over an extended period of time. Performing time-lapse imaging can allow scientists to interrogate changes in a cell or group of cells from a temporal and spatial standpoint using microscopy. In addition, maintaining cell health during these intervals is vital for the collection of data. When combined with the Invitrogen™ EVOS™ Onstage Incubator, time-lapse imaging can easily show cell migration, division, and wound healing for extended periods of time while maintaining the cells' health and function.

Using tiling and stitching scans during wound healing allows generation of an image of the entire wound. By saving the scan protocol on the EVOS FL Auto Imaging System, researchers can go back and scan the same area to observe the progress of the entire wound as it heals. The area of the fresh wound is then calculated using the measure analysis software on the EVOS FL Auto Imaging System.



In this application note, the EVOS FL Auto Imaging System is used with Gibco™ primary cells and media, Molecular Probes™ detection reagent, and the EVOS Onstage Incubator to observe cell division and migration of neonatal human dermal fibroblast (HDFn) cells in a wound healing assay over a period of 70 hours. During this time period, the cells divided and migrated to fill in the wound. This application note also shows tiling and stitching scans of an entire wound immediately after wounding and as it healed at 24 and 48 hours. Using the measure function on the EVOS FL Auto Imaging System, researchers accurately measured the area of the entire wound.

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Materials

- Gibco™ Human Dermal Fibroblasts, neonatal (Cat. No. C-004- 5C)
- EVOS Onstage Incubator (Cat. No. AMC1000)
- Invitrogen™ Molecular Probes™ CellTracker™ Red CMTPX Dye (Cat. No. C34552)
- Gibco™ Medium 106 (Cat. No. M-106-500)
- Gibco™ Low Serum Growth Supplement (Cat. No. S-003-10)

Methods

Cell growth and conditions: HDFn cells were grown in 35 mm 6-well plates to confluency and labeled with 1 µM CellTracker Red CMTPX. A scratch wound was made in the cells with a 20 µL pipette tip.

Time-lapse imaging: Imaging was performed in the Texas Red™ channel every 2 hours over a period of 70 hours with a 10x objective using the time-lapse function of the EVOS FL Auto Imaging System with EVOS Onstage Incubator.

Tiling and stitching scan: Scans of the entire wound were imaged immediately after wounding and at 24 and 48 hours with a 4x objective using the scan function of the EVOS FL Auto Imaging System. The total area of the wound was calculated using the measure function.

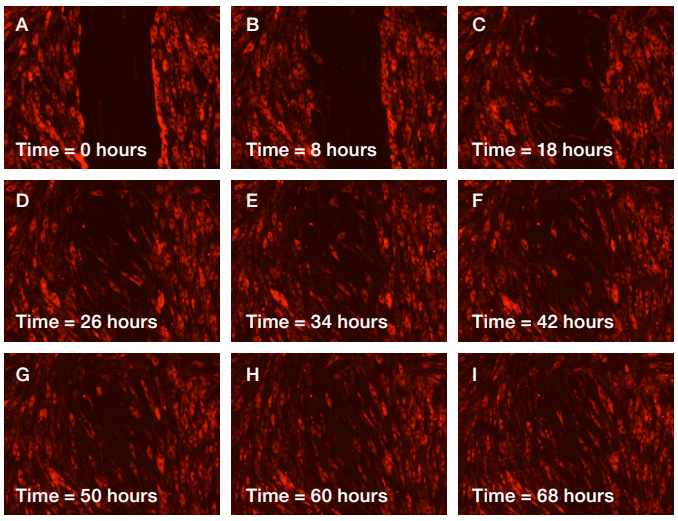

Figure 1. HDFn cells divide and migrate to fill in a wound. To see the full time-lapse movie, visit **thermofisher.com/evosflautogallery**

Results and discussion

In this experiment, a large scratch wound is clearly visible in the confluent sheet of HDFn cells (Figure 1A and 2B). By tiling and stitching a series of images (taken with the 4x objective) together, a complete view of the wound can be analyzed and measured in one image. Using the measure function of the EVOS FL Auto Imaging System, the fresh wound area was measured to be 1.82 x 10^7 µm^2 (Figure 2A). As time progresses, the remaining cells divide and migrate to fill in the wound (Figure 1B through 1H; Figure 2C) until the wound is entirely healed over with new healthy cells (Figure 1I and 2D). Using the time-lapse imaging and image tiling and stitching functionality of the EVOS FL Auto Imaging System allows researchers to effectively analyze wound healing events with and without drug treatments.

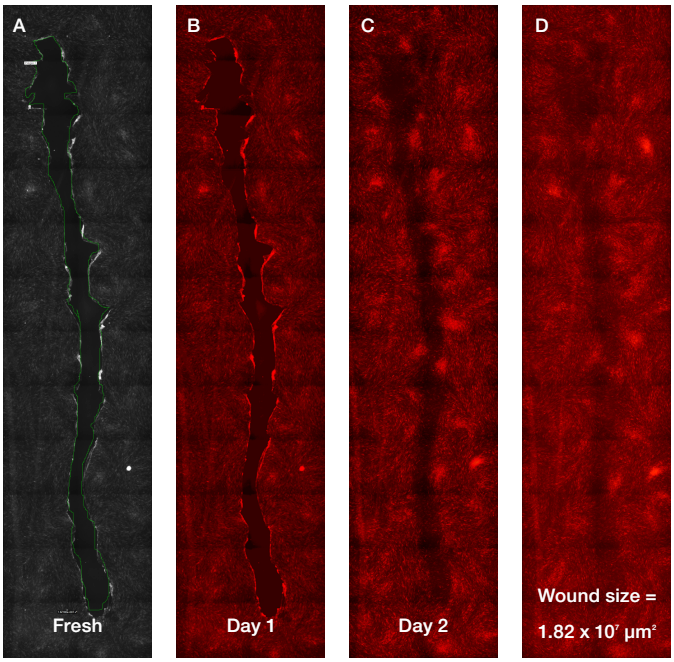

Figure 2. The area of a wound is calculated from a scan and the wound's healing progress is observed over time. A series of 24 images collected with the 4x objective was stitched together to create the images above.

Find out more at **thermofisher.com/evosflauto**

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