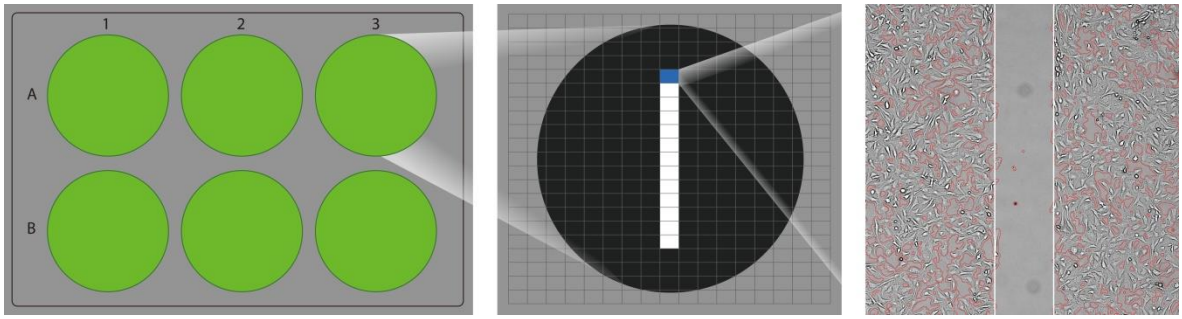
Wound healing analysis based on image segmentation

INTRODUCTION

Collective cell migration is the coordinated movement of a group of cells that maintain intracellular connections and is crucial to various biological processes including embryo development, the immune response, and cancer metastasis. The wound healing assay, also known as the scratch assay, is a simple and cost-effective way to measure collective cell migration *in vitro*. A uniform 'wound' or 'scratch' is created on a cell monolayer and the migration of the cells around the wound edge toward the cell-free space is observed via time-lapse imaging. Here, we describe a way to perform a multi-well plate wound healing assay that produces quantitative data using the CELENA® X High Content Imaging System.

APPLICATION

Bovine aortic endothelial cells (BAEC) were counted with the LUNA-II™ Automated Cell Counter, plated in a 6-well plate (SPL, 30006) at a density of 1 x 10^6 cells/well, and cultured for 24-48 hours until confluent. Scratches were made using SPLScar Scratcher (SPL, 201906). The plate was placed in the CELENA® X onstage incubator with 95% humidity and 5% CO_2 at 37°C and automated kinetic imaging was set up with the CELENA® X High Content Imaging System. Cells were imaged at the start of the experiment (t=0) and at two-hour intervals for 16 hours. 10 consecutive fields-of-view were captured per well using a 4X LWD objective and image-based autofocusing.



To quantify wound closure over time, images captured at the same time point were processed with CELENA® X Cell Analyzer software. The strategy to quantify wound closure is based on distinguishing cells from the background and measuring the change in the occupied surface area. A pipeline was created in Cell Analyzer to automatically batch process and analyze images. To do this, the *EnhanceEdges* module was used to create binary images that distinguished the foreground (the cells) from the background. The *Smooth* module was used to reduce intensity irregularities, which homogenized the cells, smoothed edges, and removed debris from the background. The resulting segmented areas were identified as cells using the *IdentifyPrimaryObjects* module, making sure that declumping was not selected so that the cell mass, not individual cells, would be identified. The identified areas were then measured using the *MeasureImageAreaOccupied* module to quantify the surface area occupied by the cells within the field. The *OverlayOutlines* module was used to overlay the original brightfield images with the outlines of the segmented areas to visually confirm segmentation precision.

Figure 1 shows one field-of-view at each time point, demonstrating wound closure over time. Evidence of cell migration is seen as early as 2 hours post-wound creation and the cell-free space steadily decreases over time as the cells move to close the space completely. Image segmentation allowed quantification of the surface area occupied by cells (Figure 2).

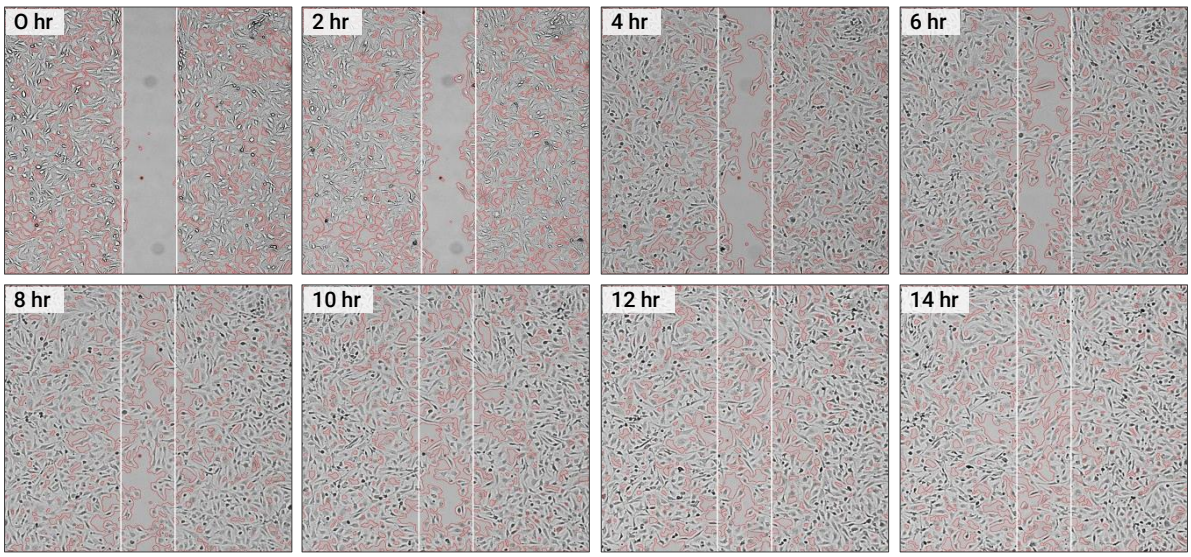


Figure 1. *Image analysis of BAEC cells showing wound closure over time. Red borders illustrate the separation between background and the areas covered by intact cells. The white boxes outline the initial wound site.*

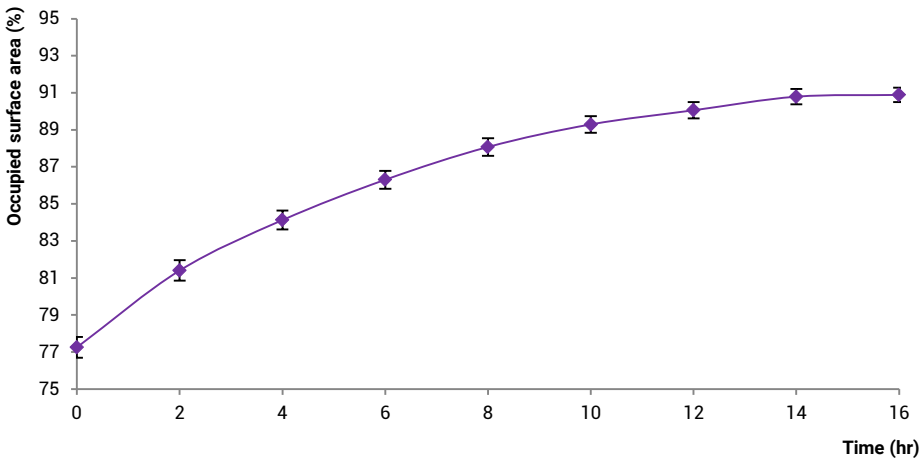


Figure 2. *Quantification of* in vitro *wound healing in BAEC cell images deduced from surface area occupied at the start and end of the experiment. N = 6 wells, 10 fields-of-view per well*

CONCLUSION

In this study, we defined a method to measure cell migration using the CELENA® X High Content Imaging System. Kinetic live cell imaging combined with quanitifcation of the change in cell surface area provides an easy-to-use, reproducible, and objective method to generate accurate cell migration data. Batch processing images for image segmentation analysis on Cell Analyzer is faster and more accurate than traditional image analysis methods. The method presented here can be modified for a variety of diverse experimental conditions and different multi-well plates, making it a high-throughput method for applications such as drug screening.

REFERENCES

Cory G. 2011. Scratch-wound assay. Methods Mol Biol. 769: 25-30.

De loso ML, Pei JV. 2018. An accurate and cost-effective alternative method for measuring cell migration with the circular wound closure assay. 38(5): BSR20180698.

Grada A, Otera-Vinas M, Prieto-Castrillo F, Obagi Z, Falanga V. Research techniques made simple: Analysis of collective cell migration using the wound healing assay. 137(2): e11-e16.

Scianna M. 2015. An extended Cellular Potts Model analyzing a wound healing assay. Comput Biol Med. 62: 33-54.