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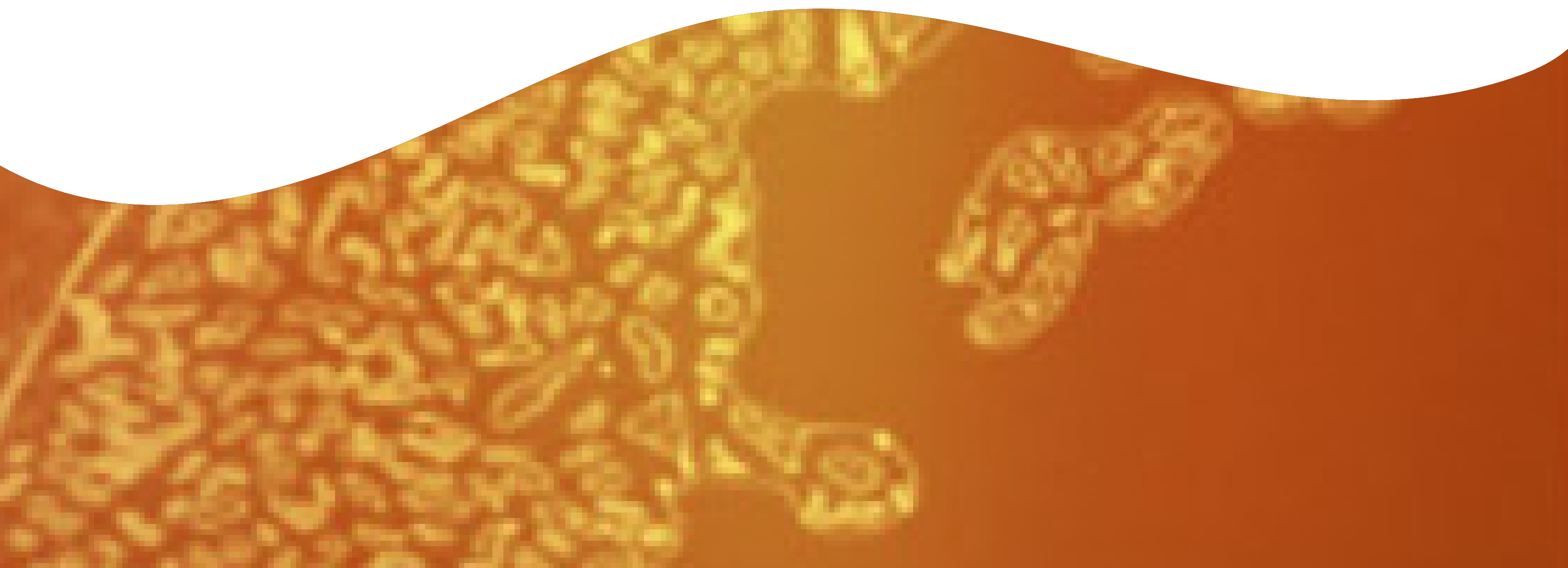
APPLICATION NOTE

Measuring wound healing and cell migration using Celigo imaging cytometer

Introduction

Cell migration is a multi-stepped, highly complex process that is involved in normal processes of cell proliferation and homeostasis, but also is exaggerated in the pathologies of metastasis and tumour invasion [1, 2]. The coordination of events has been studied at the molecular, biochemical and biophysical level for nearly 50 years. One assay which has been used throughout is the wound-healing or scratch assay. Simply defined, a monolayer of cells are grown and a border is introduced either by scratching through this monolayer to create a wound or by removing a physical barrier. The movement of cells over the margin and into the newly created space is measured. Additional information that can be gleaned from this type of assay may also be cell morphology and polarity. This biological process can substantially differ depending on the origin of the cells, the matrix they are grown on, the composition of the media and any compounds/nucleic acids that may be added as part of a screen, therefore it is important to set up a robust assay that will allow for many modifications.

The Celigo® cytometer provides a tool to measure direct *in-situ* cell migration for label-free or fluorescently labeled cells in a low, medium or high-throughput environment and can be run in a variety of multi-well plates, 6 to 96-well formats. The entire well can be imaged and a boundary (Well Mask Shape) defined which can be either circular or square, depending on shape of the user's template. The system can then measure cell number or % confluence of cells within the previously cleared region.



As the Celigo does not require fixing of cells, multiple images/well/plate can be taken and a time course can be plotted (Wound Healing Growth Tracking Report). The Wound Healing Application uses the Celigo's rapid scanning capability combined with intuitive software to follow cell migration. In addition, images may be reanalyzed using the software to extract additional data such as cell morphology which may allow the user to interpret migration or invasion.

Approach and results

To demonstrate the use of the Celigo cytometer for the measurement of Wound Healing, we used the Oris™ Cell Migration Assay (Platypus Technologies, LLC), cultured the cells with increasing concentrations of Cytocholasin-D to inhibit actin polymerization and cell migration [3], and measured cell migration in 2-hour increments for 48 hours. Confluency and cell counts were obtained at each timepoint and growth curves for each well were created. Data was exported and a Cytocholasin-D dose-response curve was generated.

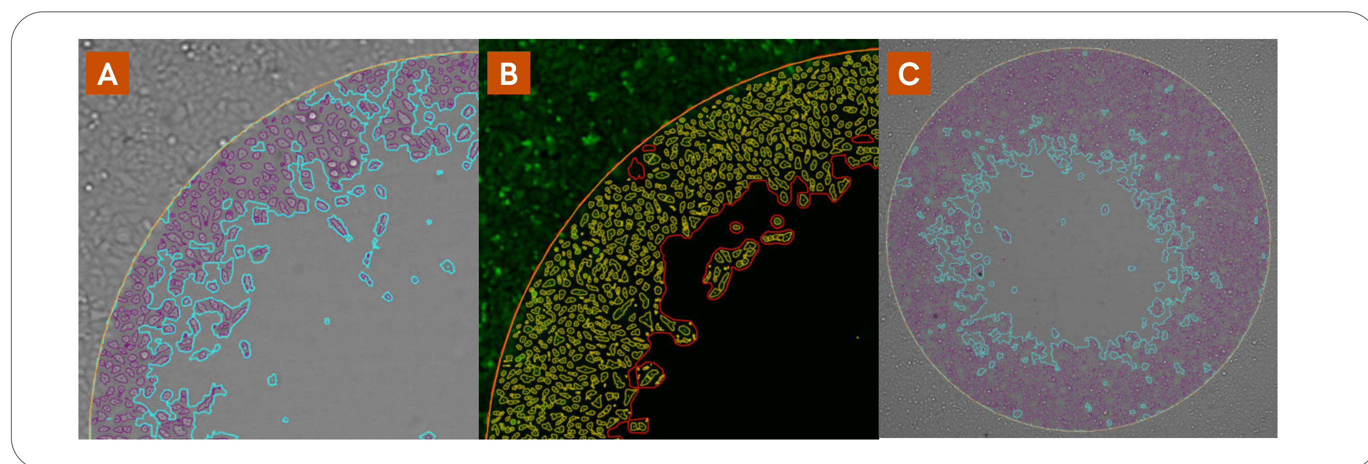


Figure 1. 60x10^3 Human sarcoma cells (HT-1080) were plated into each well of an Oris™ 96-well plate allowed to grow overnight at 37 °C. Silicone stoppers were removed to introduce a 2mm round detection zone within each well. (A) Brightfield image showing partial well segmentation of individual cells (purple outline) as well as confluence using a texture algorithm (blue outline). The flexible well mask was set to 40% of well (yellow line) defining the region of migration. (B) HT-1080 Cells stained with Cell Tracker Green (Invitrogen). Individual cells (yellow outline) as well as confluence border (red outline) can be identified. The well mask set to 40% of well (orange line). (C) Whole well image showing boundary of cell migration as well as individual cells and Confluence outline.

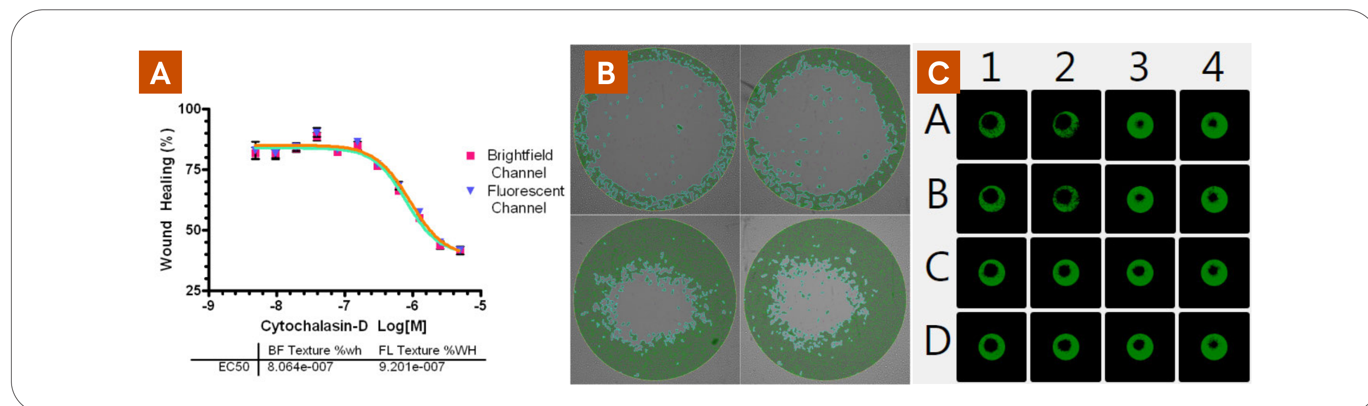
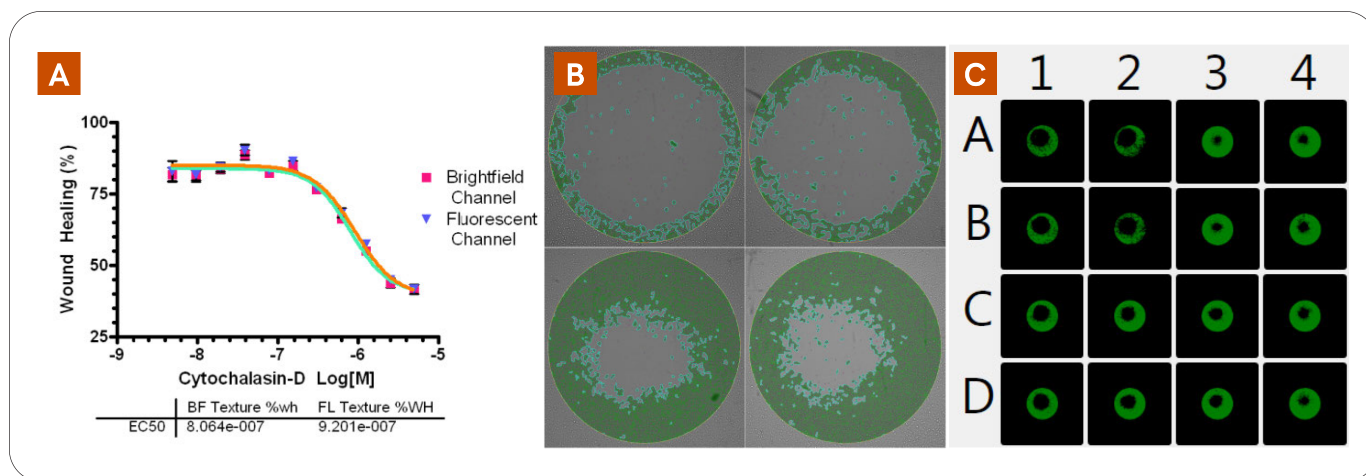


Figure 2. The Celigo cytometer was used to calculate a dose response curve of Cytochalasin-D on the HT-1080 cells. After cells were seeded onto Oris™ assay plates and allowed to adhere overnight, the stoppers were removed, media was aspirated and replaced with growth media or growth media containing drug concentrations from 5 – 0.01 µM. (A) Brightfield and Fluorescent measurements of % Wound Healing were taken at 24 hours. Data was exported as well-level CSV and imported into Prism to determine EC50's within the SD. (B) Whole well images of positive control (top wells) and negative control (bottom wells) HT-1080 cells showing inhibition of wound healing and normal migration, respectively. Brightfield confluence images (light blue outline) are filled to allow visualization of monolayer. (C) Thumbnail images of filled, masked images (area of migration) seen from Results Tab with Celigo 2.1 software. Wells A1-B2 have highest concentration of drug, A3-B4 have no drug and C1-D4 have varying concentrations of drug.



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Conclusions

This application note demonstrates the use of the Celigo cytometer to measure Wound Healing in an automated, highly reproducible manner. Results that were generated were consistent using either brightfield or fluorescent imaging. The system allows great user flexibility by allowing different segmentation settings to be used for specific cell-types.

The user can choose from many plate-well formats and the software allows for the user to decide which type of wound to create by allowing either a round or square mask that can be adjusted based on % well filled. Using this system will allow the user to generate data rapidly and reliably with or without labeling the cells. Finally, scans can be reanalyzed using Celigo's other applications such as cell counting to obtain specific morphological data or plates can be rescanned with other applications to measure cell health, viability or cell cycle.

References

1. Maria Grazia Lampugnani (1999), "Cell Migration into a Wounded Area *In Vitro*", Adhesion Protein Protocols: Methods in Molecular Biology, 96:177-182
2. Justin C. Yarrow, Zachary E. Perlman, Nicholas J. Westwood, and Timothy J. Mitchison (2004), "A high-throughput cell migration assay using scratch wound healing, a comparison of image-based readout methods", BMC Biotechnology, 4:21
3. Manfred Schliwa (1982), "Action of Cytochalasin D on Cytoskeletal Networks", Journal of Cell Biology, 92(1):79-91

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