A novel image-based cytometry analysis for measuring cell migration in wound healing assay

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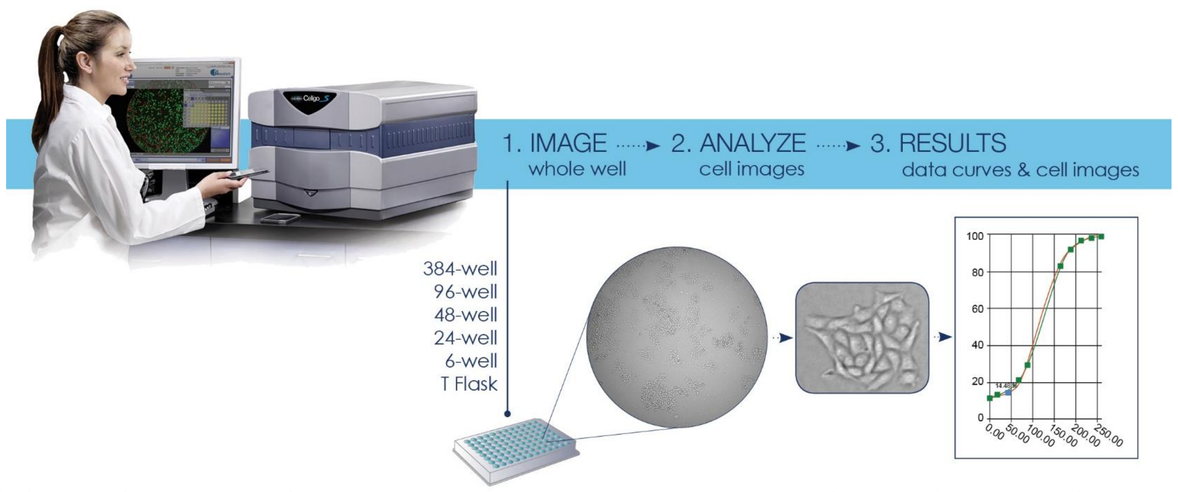
1. ABSTRACT

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. 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. In this work, the Celigo Imaging Cytometer was used to measure direct *in-situ* cell migration for label-free or fluorescently labeled cells for cell migration analysis. 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, and measured cell migration in 2-hour increments for 48 hours. Confluency and cell counts were obtained at each time-point and growth curves for each well were created. Data was exported and a Cytocholasin-D dose-response curve was generated. The Celigo Imaging Cytometer generated highly reproducible cell migration results, as well as consistent data using either bright field or fluorescent imaging. The Celigo software allows different plate selection and analysis methods for the researchers to optimize their analysis parameters, which can generate more accurate cell migration results. The Celigo Imaging Cytometer can be an essential tool for researchers to greatly improve accuracy of results and efficiency.

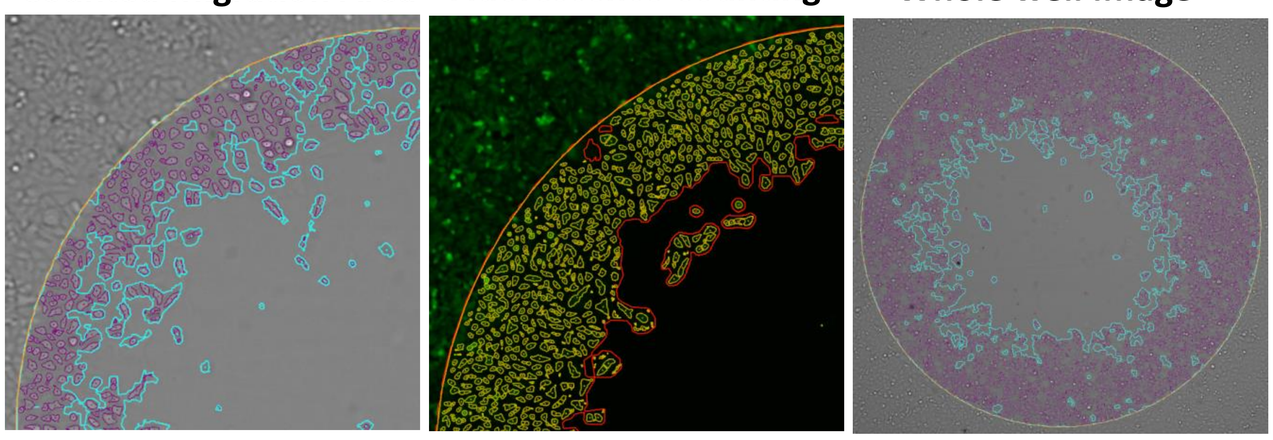
2. CELIGO IMAGING CYTOMETRY FOR WOUND HEALING ASSAY



1. Celigo Imaging Cytometer is a plate-based cytometer that can scan the entire well of standard microplates and captures bright-field and fluorescent images
2. The captured images are analyzed with the Celigo software to measure size, morphology, cell count, confluence, and fluorescent intensity
3. The measured parameters are used to generate cell proliferation kinetic data, GFP/RFP expression, tumor spheroid size change, DNA cell cycle analysis, apoptosis, and ADCC cytotoxicity results

3. TRADITIONAL VS CELIGO ASSAY PROTOCOL

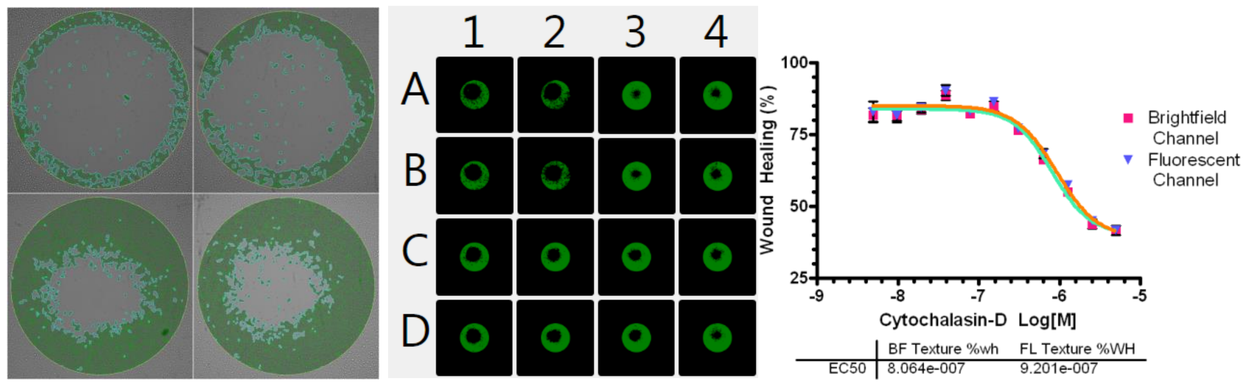
Counted Migration Area | **Fluorescence Counting** | **Whole well Image**



1. 6 x 10^4 Human sarcoma cells (HT-1080) were plated into each well of an Oris™ 96-well plate allowed to grow overnight at 37°C
2. Silicone stoppers were removed to introduce a 2 mm round detection zone within each well
3. Bright field image (left) shows the partial well segmentation of individual cells (purple outline) as well as confluence using a texture algorithm (blue outline)
   a) The flexible well mask was set to 40% of well (yellow line) defining the region of migration
4. HT-1080 Cells stained with Cell Tracker Green (Invitrogen) are shown in the fluorescence (middle), where individual cells (yellow outline) as well as confluence border (red outline) can be identified
   a) The well mask set to 40% of well (orange line)
5. The whole well image shows the boundary of cell migration as well as individual cells and Confluence outline (right)

4. WOUND HEALING TRACKING OF HT-1080 CELLS WITH CYTOCHALASIN-D

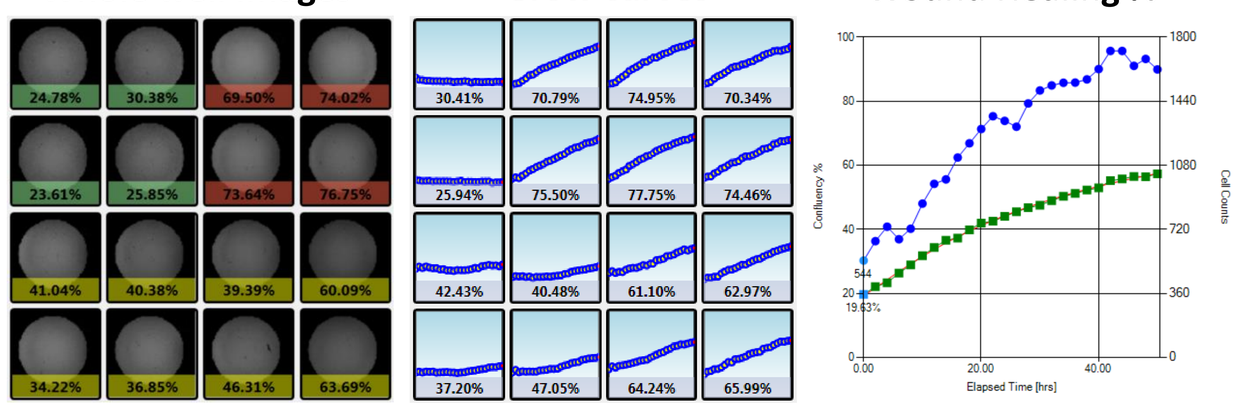
Counted Migration Area | **Filled View** | **Wound Healing %**



- The Celigo™ image 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
- Whole well images (left) show the effect of positive (top wells) and negative control (bottom wells) on the inhibition of wound healing and normal migration, respectively
  - Bright field confluence images (light blue outline) are filled to allow visualization of monolayer
- Filled view (middle) of thumbnail masked images (area of migration) are from Results Tab with Celigo™ 2.1 software
  - Wells A1, A2, B1, B2 have highest concentration of drug, A3, A4, B3, B4 have no drug, and C1-C4, D1-D4 have varying concentrations of drug
- Bright-field and Fluorescent measurements (right) 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

5. WOUND HEALING IMAGES AND KINETIC TIME PLOTS

Whole well images | **Grow curves** | **Wound Healing %**



- A wound healing time course was generated by scanning plate in Celigo™ Cytometer every two hours for 48 hours following addition of drug and created within the Celigo 2.1 software
  - Each scan result is added to the database as a separate time-point
- Thumbnail images of scanned wells (bright field) showing endpoint % confluence (left)
  - Thresholding is turned on and a Heat-map is generated to allow visual interpretation of results
  - This function can be turned on or off and the threshold is manually entered.
- Thumbnail images of growth curves showing % Confluence for each well in 96-well plate using Celigo Growth Tracking Report function (middle)
- Single well growth curve showing Confluency % and Cell Count in one well of 96-well plate (right)

6. SUMMARY AND CONCLUSION

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 bright field 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

- Maria Grazia Lampugnani (1999), "Cell Migration into a Wounded Area In Vitro", Adhesion Protein Protocols: Methods in Molecular Biology, 96:177-182
- 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
- Manfred Schliwa (1982), "Action of Cytochalasin D on Cytoskeletal Networks", Journal of Cell Biology, 92(1):79-91