SARTORIUS

Simplifying Progress

Kinetic Live-Cell Visualization and Quantification of Cell Migration

A. Ward*, J. Trigg, K. Barnes, G. Lovell, T. Dale

Sartorius UK Ltd., Royston, Hertfordshire, UK
*Corresponding author: Amber.Ward@Sartorius.com

Summary & Impact

- Cell migration is a multistep process that enables cell movement in response to an environmental stimulus, this plays a role in physiological and pathological processes, including wound healing and tumor metastasis.
- The rapid rearrangement of actin filaments leads to a cycle of leading edge protrusion and lagging edge retraction, summating in the associated cellular morphological changes.
- Here we exemplify an assay for the visualization and kinetic assessment of cell migration and its modulation in a 96-well plate. Incucyte® Live-Cell Analysis enables automated image-based measurements of cell migration *in vitro* via label-free or dual fluorescence readouts.
- To simultaneously monitor cell cycle dynamics, cells expressing the Incucyte® Cell Cycle Lentivirus Reagent were used. Images were acquired using the Incucyte® Live-Cell Analysis System and analyzed using integrated software.
- These data demonstrate that live-cell analysis methods provide a solution to robustly measure cell migration in real time without relying on end point analysis.

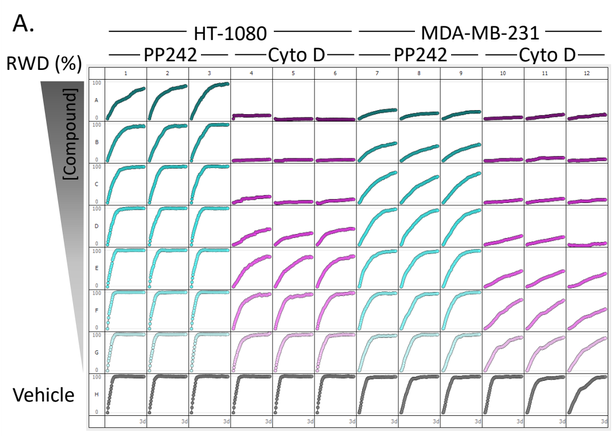
Incucyte® Live-Cell Imaging and Analysis Solutions

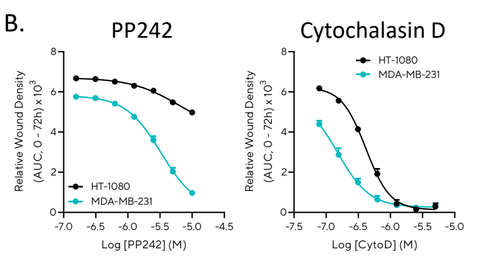
Incucyte® Live-Cell Analysis System
A fully automated phase contrast and multi-color fluorescence system that resides within a standard cell incubator for optimal cell viability. Designed to scan plates and flasks repeatedly over time.

Incucyte® Software
Fast, flexible and powerful control hub for continuous live-cell analysis comprising image acquisition, processing and data visualization.

Incucyte® Reagents and Consumables
A suite of non-perturbing cell labelling and reporter reagents. Includes nuclear-targeted fluorescent proteins for cell counting plus no-wash cell health reagents for apoptosis, cytotoxicity, and many more.

Pharmacological assessment of inhibitors of cell migration





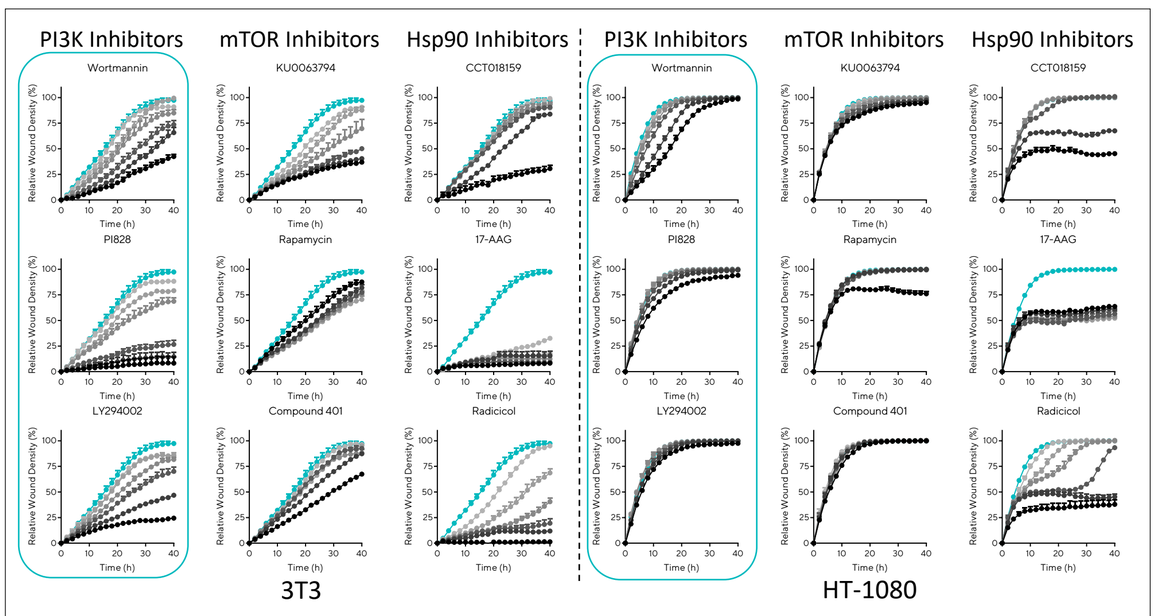
Cell type	Compound	IC_{50} (µM)	95% CI (µM)
HT-1080	PP242	3.97	3.57 - 4.43
	Cytochalasin D	0.42	0.39 - 0.46
MDA-MB-231	PP242	2.61	2.4 - 2.84
	Cytochalasin D	0.22	0.19 - 0.24

- HT-1080 and MDA-MB-231 cells were seeded at 30K/well and left for 24 hours to form confluent monolayers. Cells were wounded using the Woundmaker Tool and treated with compounds.

A. 96-well microplate graph shows relative wound density (RWD) over 72 hours, which enables visualization of cell migration in the presence of inhibitory compounds PP242 (mTOR inhibitor) and cytochalasin D (actin polymerization inhibitor).

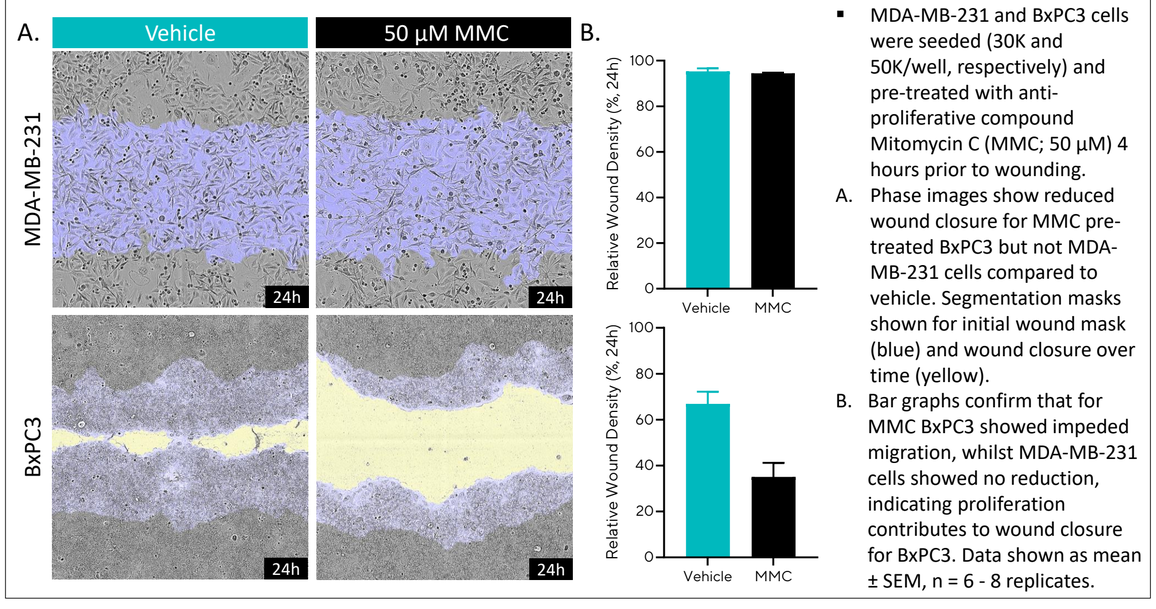
B. Concentration response curves indicate the efficacy of each compound in both cell types with IC_{50} values and confidence interval (CI) shown (table). Data shown as mean ± SEM, n = 3 replicates.

Cell- and molecule-dependent cell migration profiles



- 3T3 and HT-1080 cells (30K/well) were wounded and treated with a concentration-range (0.1 – 30 µM) of 9 inhibitors of PI3K, mTOR (both associated with Akt pathway), and Hsp90 (chaperone protein for stability).
- PI3K inhibitors showed a significant difference in efficacy with HT-1080 appearing more resistant than 3T3 cells. HT-1080 have an activated n-RAS oncogene (upstream activator of PI3K-Akt pathway) which via sustained signaling, may contribute to observed resistance. Data shown as mean ± SEM, n = 3 replicates.

Anti-proliferative effects of mitomycin C during cell migration

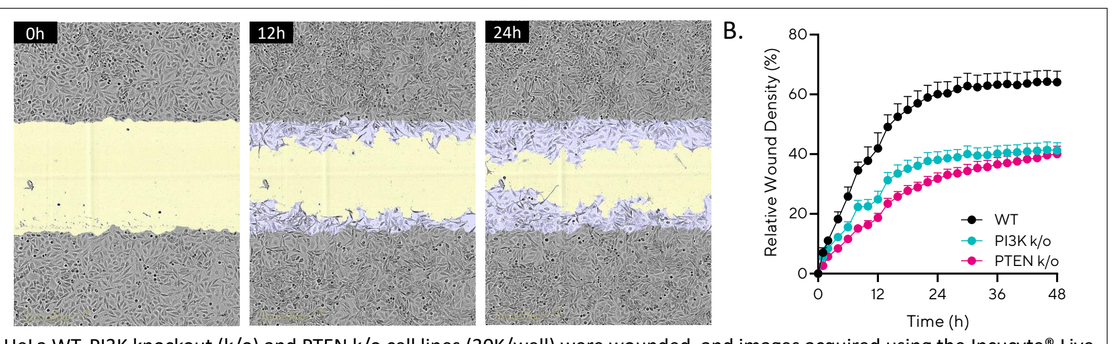


- MDA-MB-231 and BxPC3 cells were seeded (30K and 50K/well, respectively) and pre-treated with anti-proliferative compound Mitomycin C (MMC; 50 µM) 4 hours prior to wounding.

A. Phase images show reduced wound closure for MMC pre-treated BxPC3 but not MDA-MB-231 cells compared to vehicle. Segmentation masks shown for initial wound mask (blue) and wound closure over time (yellow).

B. Bar graphs confirm that for MMC BxPC3 showed impeded migration, whilst MDA-MB-231 cells showed no reduction, indicating proliferation contributes to wound closure for BxPC3. Data shown as mean ± SEM, n = 6 - 8 replicates.

Quantification of cell migration profiles in real time

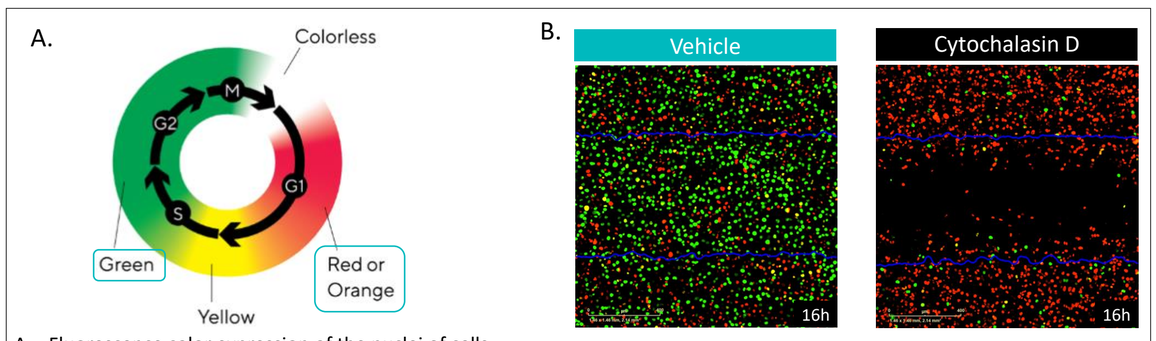


- HeLa WT, PI3K knockout (k/o) and PTEN k/o cell lines (30K/well) were wounded, and images acquired using the Incucyte® Live-Cell Analysis System.

A. Phase images of HeLa WT cells. Segmentation shows the initial (blue) and wound closure over time (yellow).

B. Quantification revealed differential rates for WT vs k/o cells with migration plateauing by 48 hours in all cell lines. Data shown as mean ± SEM, n = 3.

Cytochalasin D inhibits cell cycle and migration

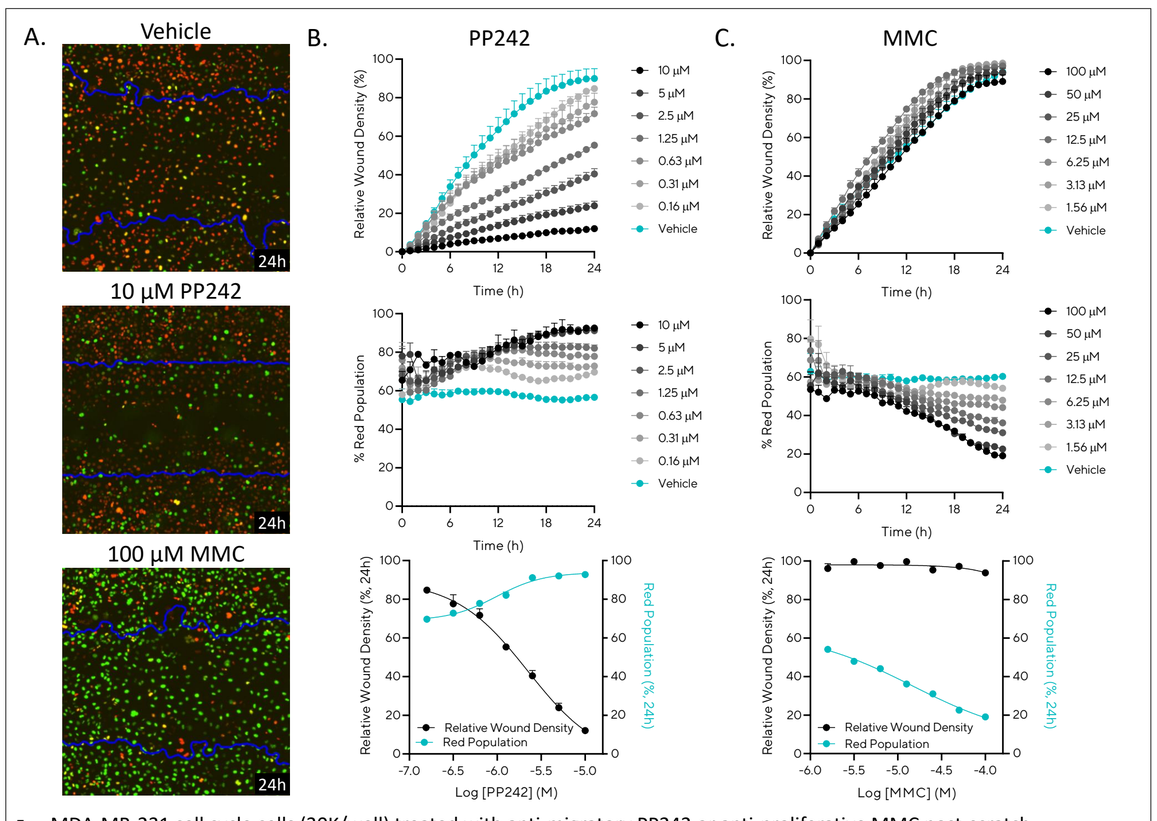


A. Fluorescence color expression of the nuclei of cells expressing the Incucyte® Cell Cycle Green/Red Lentivirus.

B. HT-1080 cell cycle cells were seeded (30K/well), wounded and treated with 0.31 µM cytochalasin D. Fluorescence images with initial scratch outline (blue) reveal cell cycle arrest in G1 (red) compared to vehicle.

C. Time-courses show cytochalasin D inhibits cell migration (RWD) and arrests cell cycle in G1 (Red Population), calculated by expressing red count as a percentage of the summed red and green count within the wound). Data shown as mean ± SEM, n = 3 replicates.

Differential compound effects on cell cycle and migration



- MDA-MB-231 cell cycle cells (30K/well) treated with anti-migratory PP242 or anti-proliferative MMC post-scratch.

A. Fluorescence images enable visualization of cell migration (initial scratch outline in blue) and cell cycle arrest for treated and vehicle conditions.

B. PP242 had a concentration-dependent effect on MDA-MB-231 by inhibiting both cell migration (relative wound density) and the cell cycle, with an increase in cells in G1 within the wound (Red Population).

C. In contrast, MMC had no little-to-no effect on migration but had a concentration-dependent effect on the cell cycle with an arrest in S/G2/M (green) as shown by a decreasing red population. Data shown as mean ± SEM, n = 3 replicates.