High-fidelity 96-well kinetic imaging assays for cell migration

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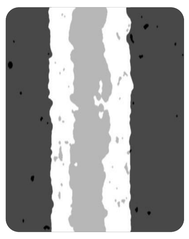
Summary & Impact

- Cell migration is a pivotal event in a range of physiological and pathological processes including inflammation, wound healing & tumour development
- We have evolved the well established scratch wound assay of cell migration into an image-based, facile, robust, **fully kinetic** 96-well paradigm
- The approach is amenable to a range of cell types and screening of small molecules, biologics and gene-interference reagents (e.g. siRNA, miRNA)
- Our solution yields hitherto uncharacterized and information-rich temporal differences in the profile of modulation by different pharmacological agents

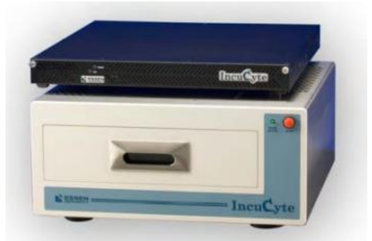
96-well Scratch Wound Assay – an integrated solution

96-Well WoundMaker

Image Lock Plates



Mask algorithm



IncuCyte live cell imager

Experimental protocol

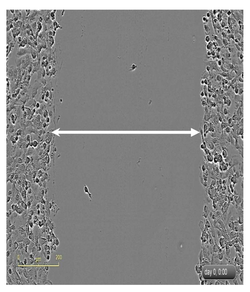
- Seed cells onto 96-well image lock plate & grow to confluence
- Create wound in all wells using 96-well woundmaker (1 min). Wash x3 & add test compounds
- Place in IncuCyte live-cell imager and gather 'HD'-phase images every 1-6h until wound has 'healed' (up to 6 plates at once)

Analysis

- IncuCyte software automatically processes images & quantifies migration (e.g. time vs wound width plot)
- High quality, time-lapse videos from each well can be easily created to visualise migration
- Facile data exports to other software for summary analyses (e.g. concentration response curves)

Highly consistent wounds & migration profiles

A



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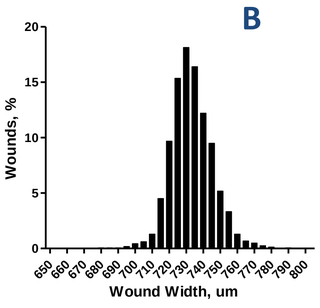
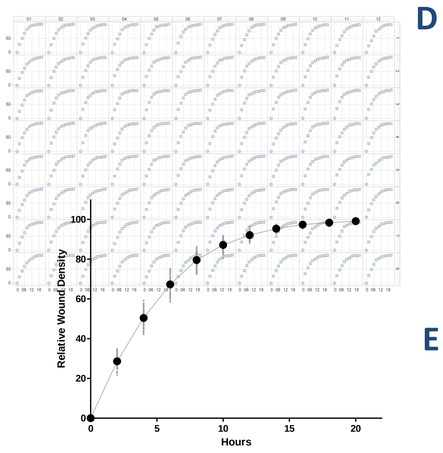


D



C

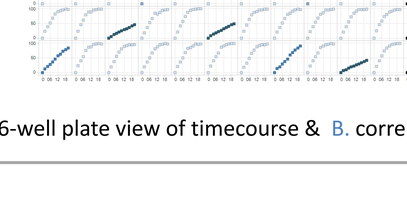
Cell Type	Initial Wound Width (microns)	CV of Initial Wound Width	Initial Wound Confluence
HT 1080	733	1.7 %	0.6%
NIH 3T3	734	2.1%	0.3%

E

A. Phase contrast image of representative 'wound'
B. Histogram showing consistency of wound widths
C. Summary table of wound width CVs in different cell types
D. 96-well plate view of timecourse of cell migration into wounded area (t *vs* relative wound density)
E. Mean timecourse (HT1080 cells)

Temporal pharmacology – Cytochalasin D

A



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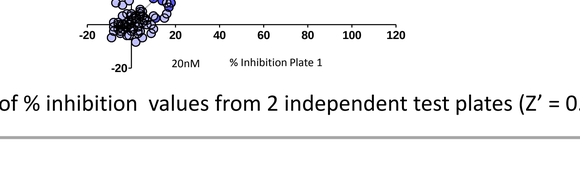


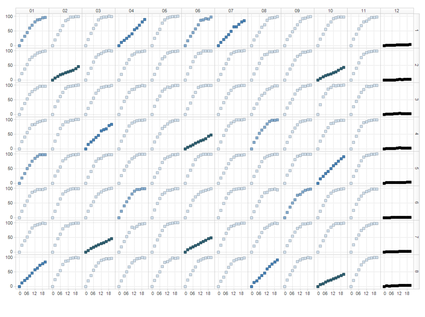
C

	Cyto D pIC_{50} at different assay end points									
Replicate	2h	4h	6h	8h	10h	12h	14h	16h	18h	20h
1	6.7	6.7	6.8	6.8	6.7	6.6	6.6	6.5	6.5	6.4
2	7.1	6.9	6.9	6.8	6.7	6.6	6.5	6.4	6.4	6.4
3	6.8	6.8	6.8	6.7	6.6	6.6	6.5	6.5	6.4	6.4
4	6.6	6.5	6.5	6.5	6.5	6.4	6.4	6.4	6.4	6.3
5	8.2	7.1	6.9	6.8	6.7	6.5	6.5	6.4	6.4	6.4
6	6.8	6.8	6.8	6.8	6.7	6.6	6.6	6.5	6.5	6.4
10	7.0	6.9	6.9	6.8	6.7	6.6	6.5	6.5	6.4	6.4
11	-	7.5	7.0	6.7	6.6	6.5	6.5	6.4	6.4	6.4
12	7.2	7.1	7.0	6.8	6.7	6.6	6.5	6.5	6.5	6.4
Average	7.0	6.9	6.8	6.7	6.7	6.6	6.5	6.5	6.4	6.4
SD	0.51	0.29	0.15	0.10	0.08	0.07	0.06	0.05	0.04	0.03

A. 96-well plate view of timecourse of cell migration in the absence and presence of increasing concentrations of cytochalasin D
B. Average data of timecourse & concentration-response curves
C. Summary table of time-dependence of IC_{50} values

Single shot screening – spiked plate Cytochalasin D

A



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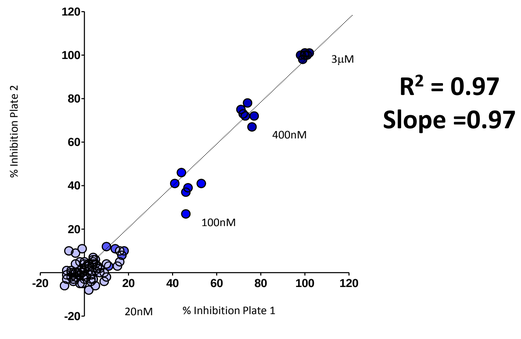
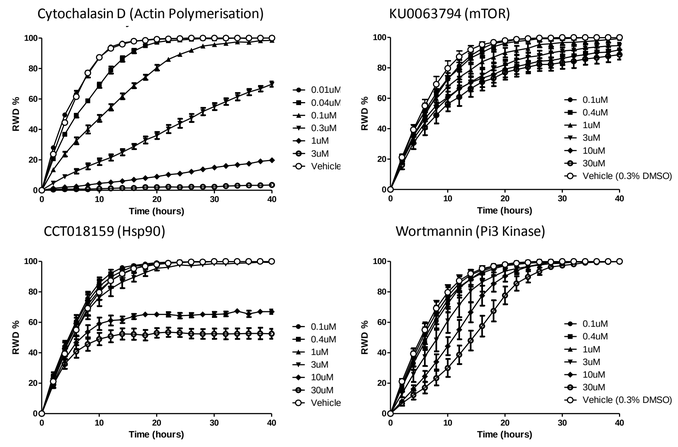


$R^2 = 0.97$
Slope =0.97

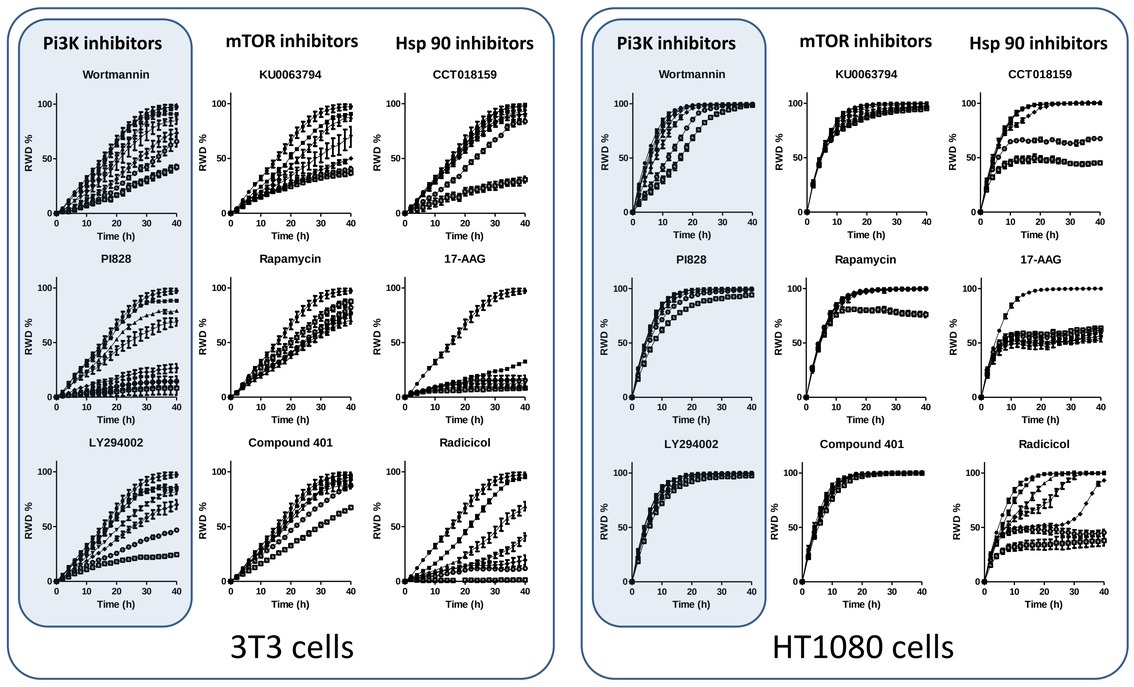
A. 96-well plate view of timecourse & B. correlation of % inhibition values from 2 independent test plates (Z' = 0.9)

Cell signalling inhibitors yield different kinetic profiles



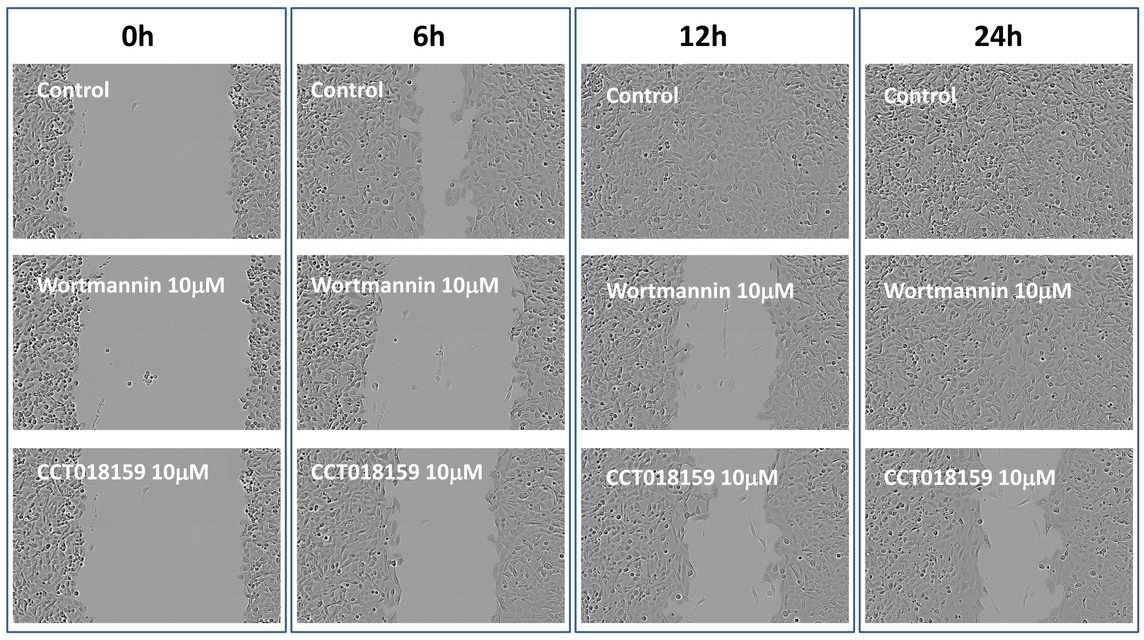
- Average time course data from HT1080 cells
- Compounds added to cells immediately after wound creation (t=0)
- Data from n=4 96-well plates
- Note immediate attenuation of migration by wortmannin (lower right), consistent with a role of PI3K in defining cell polarity and the leading edge
- Note attenuation of later phases of migration by CCT018159 (Hsp90 inhibitor)

Temporal profiles: cell type- and molecule- dependent



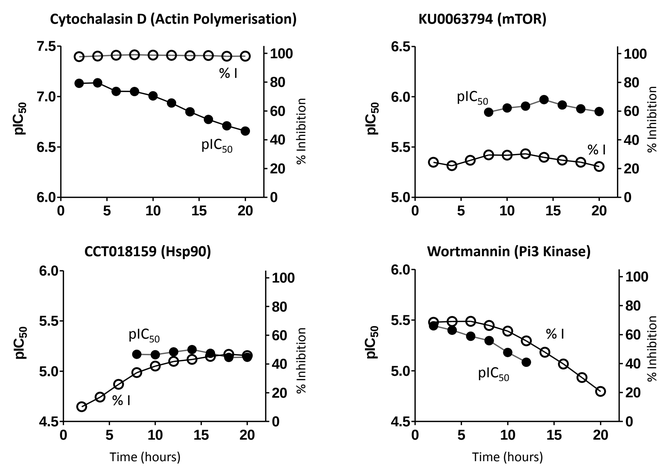
e.g. mIgration of 3T3 cells are more sensitive to attenuation by PI3 kinase inhibitors than HT1080 cells

Data validation: time-lapse image analysis



Phase-contrast images (HT1080 cells, 20x) from wells taken at different time points, with different inhibitors. With HD optics and time lapse analysis, cell morphology and migratory movements can be readily tracked

Small molecule potency & efficacy: temporal profiles



- HT1080 cells, n=4
- Potency (pIC_{50}) and efficacy (% max inhibition) values were obtained at different time points from curve fits to the concentration-response data.
- Note **decreasing** potency and efficacy of wortmannin with time
- Note i**ncreasing** potency of CCT018159 with time