Wound Healing Dashboard

Summary of the metrics you can get

Scratch wound assays are used to measure the collective movement of cells. Here a scratch is formed in a confluent layer of cells leaving an empty wound. Upon wound formation cells begin to move collectively into the scratch area eventually leading to the healing of the wound and the reformation of a confluent layer. Conventional metrics used to measure this collective movement of cells involve the measurement of the wound area over time. Although this does give an overview of the relative time it takes for a wound to close, these results are highly influenced by the starting wound area as well as cell morphological changes.

Livecyte enables you to measure wound closure rates independent of wound area with collective migration, leading edge cell track speed and directionality metrics. In addition, as morphological and proliferation metrics are given as standard for every assay this can also be monitored to ensure that wound closure is due to cellular migration, not an increase in cell area or cell count.

Applications where it would be useful

Scratch wounds are used in a variety of different specialisms in biological research where the motility of cells moving as a collective needs to be quantified. This includes:

Cancer Research – where the collective movement of tumour cells indicate levels of invasiveness with more rapidly moving cells leading to higher rates of metastasis and tissue invasion.

Embryonic development – where layers of cells move together towards their programmed destination.

Wound healing – where epithelial cells move collectively into a wound bed to re-epithelialise, secrete collagen, and repair damaged tissue.

Starting Area - The area of the scratch detected in the first frame of the timelapse. This serves as a quality check when surveying the consistency of the experimental set up. This is especially relevant when utilising confluence based traditional metrics such as T1/2 and wound area over time which can be significantly affected by scratch area as cells must move more to fill larger areas.

Area over time - The sum of the area of the voids that are considered part of the scratch over time. As a wound closes the area will decrease. A steeper reduction in wound area indicates that cells are moving more quickly into the wound.

Area T½ - The time taken for the area of the scratch to halve. The halving time is fitted using a linear regression using all data where the area is above half of the starting area. This allows us to estimate a half-life if the scratch does not reach 50% of the starting area, as well as compensate should the scratch area increase above 50% after it initially went below.

Collective Migration - The collective migration is the mean rate of closure of the scratch. It is calculated by dividing the closure rate of the scratch area by twice the scratch length. This then gives the rate at which each side is migrating into the scratch. Collective Migration is a measurement independent from the area of the wound and so is not affected by variations in starting wound area and thus a more robust measurement of the bulk wound closure rate.

Track Speed - A distribution plot of the speeds of the leading-edge cells. Higher speeds indicate cells are moving faster, however not necessarily that the wound closes quicker as cells may not be moving directly into the wound.

Directionality - A rose plot of the direction of movement of the leading-edge cells. With a longer segment indicating a larger proportion of cellular movements in that direction. Movement directly towards the centre line of the scratch is represented as up for all cells in this plot those on opposite sides of the wound have their directionality mirrored. More directly moving cells often cause an increase in collective migration, and wound healing rates. This is due to cells moving more directly into the wound site.

Publication Example

Noble, A.R., Hogg, K., Suman, R. *et al.* Phospholipase D2 in prostate cancer: protein expression changes with Gleason score. *Br J Cancer* **121,** 1016–1026 (2019). https://doi.org/10.1038/s41416-019-0610-7

In this study Noble et al used Livecyte to study the effects of phospholipidase 2 (PLD2) inhibition on collective migration in primary prostate cancer cells. Primary prostate cancer monolayers were treated with PLD2 inhibitors and scratch wounds were imaged using Livecyte over 24 hrs.

Combined inhibitors caused the biggest decrease in cell track speed, and collective migration as well as the slowest wound healing rate, and almost isotropic cell directionality. The untreated control showed a strong directionality of cellular movement into the wound, and the fastest wound healing rate, collective migration, and track speed.