Watch it Live with DRAQ9™: Time-Lapse Imaging of Cell Behavior on 2D Substrates and in 3D Aggregates

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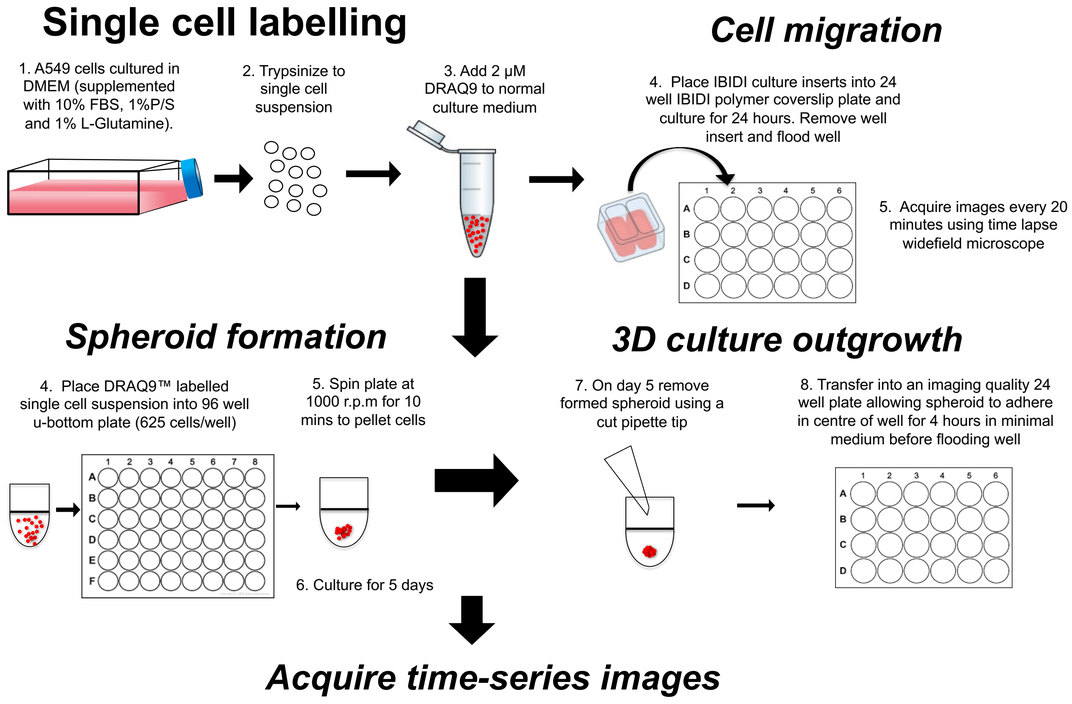
Introduction

The importance of cell migration and proliferation during critical stages of wound healing, tumour metastasis and tissue remodelling is under continued investigation. As part of these comprehensive studies tracking single cells and cell communities has become a potent tool to measure biological functional capacity and a systems response to perturbation. Delivering such a tracking capability and robust assay readouts is multi-faceted. First, it requires a cell label that is non-toxic, stable throughout, as cell identifier and locator. We describe use of a cell-permeant, far-red fluorescent, cytoplasmic probe DRAQ9™ (BioStatus) that is non-toxic, to be present continuously as a real-time tracker such that fluorescence is undiminished over time e.g. during spheroid aggregation, formation and expansion; in 2D scratch-wounds; or a hybrid assay, as single cells emerge onto 2D substrate from a spheroid. In all cases, DRAQ9™ labels cells for unique identification over time, the second key property. Thirdly, this stable tracker provides a readout ripe for automated cell tracking in aggregates, migration in 2D or indeed through mitosis.

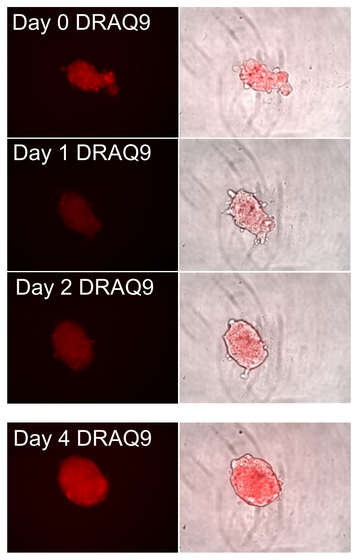
Methods

Single cell labelling

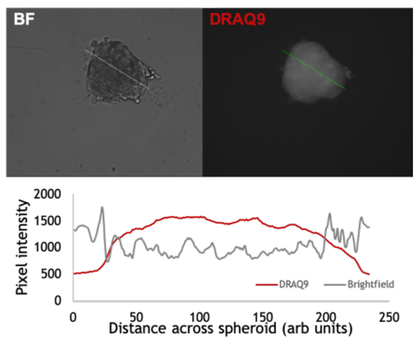


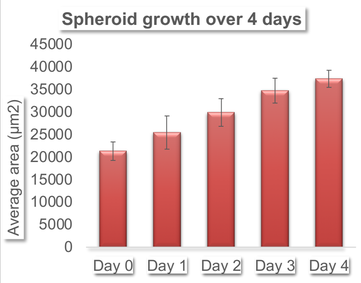
1. Watch it grow - *spheroid painting*

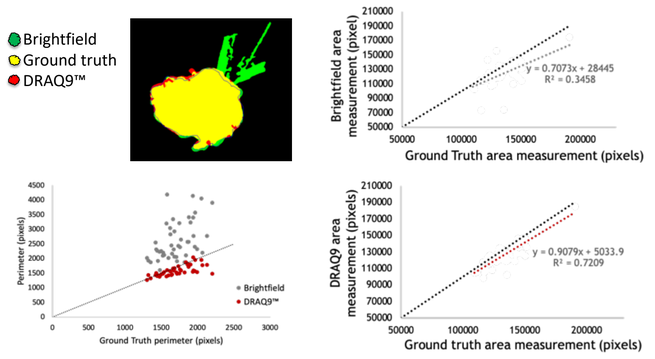
Watch it here!



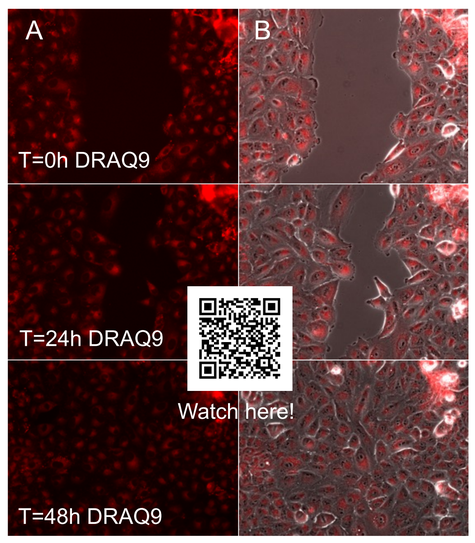
- DRAQ9™ fluorescent images used to create consistent mask for image analysis
- DRAQ9™ mask allows exclusion of artefacts present in brightfield images



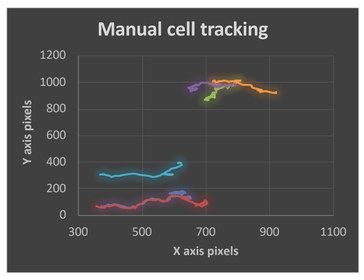




2. Watch it migrate - *single cell tracking*

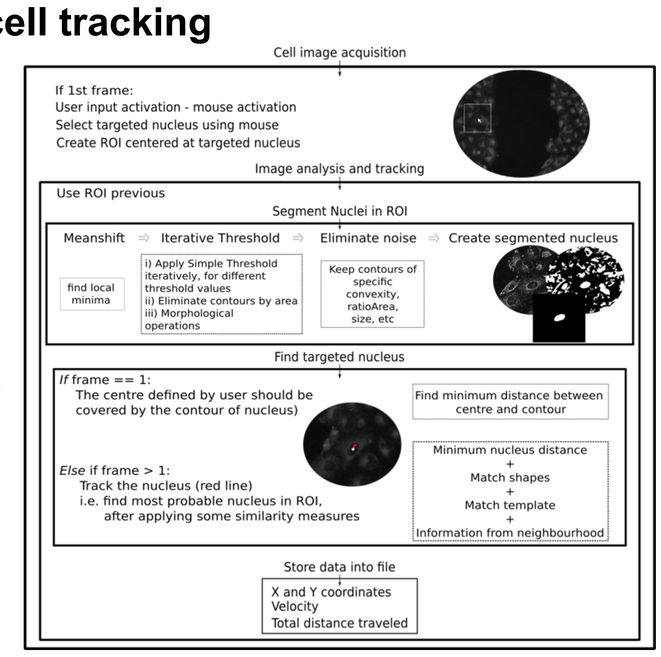


- A549 cells labelled and continuously cultured with 2 µM DRAQ9™
- Images acquired at 20 min. intervals to record mitotic events
- Cell migration observed over 24 hours
- DRAQ9™ (A) and with brightfield overlaid (B) images used to manually track cells (C)
- Gain morphometrics, and now:
  - average speed
  - displacement
  - distance travelled



Automated cell tracking

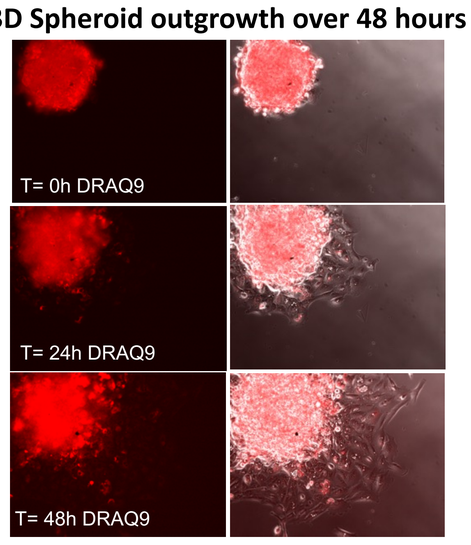
- DRAQ9™ Images used for automated tracking of cells
- Absence of nuclear staining provides consistent object segmentation
- Change in cell morphology during mitosis changes DRAQ9™ fluorescence localisation, providing a new object feature to be tracked



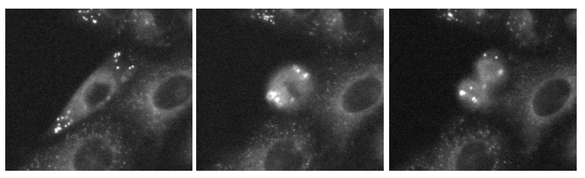
3. Watch it emerge - *3D culture studies*

Watch it here!

3D Spheroid outgrowth over 48 hours



Cellular events- mitosis



- DRAQ9™ is continuously present in the culture medium from the start so cells are stably labelled.
- Cells emerging from 3D cultures can be studied and higher magnification images can be used to visualise emerging cell behaviour

Discussion / Future Work

- DRAQ9 labelling of live cell cultures enables real-time tracking of cell migration and cell division. The visualization of cell-cell and cell-matrix interactions provides rich information on cell behaviour within a niche.
- The far-red fluorescence makes it compatible with imaging on sub-optimal imaging substrates e.g. tissue culture plastic. Changes in fluorescence intensity adds information on cellular division, allowing such events to be localised to a cell niche.
- Overlays of DRAQ9 and brightfield images make manual tracking easier to perform, increasing confidence in cell identification, especially during cell division.
- Importantly, the absence of DRAQ9 in nuclei provides a mask that can be used in automated image analysis and reduces the time required for image analysis.