Label-free ptychographic imaging reveals a role for VGSC-mediated membrane potential depolarisation in enhancing the migratory capability of breast cancer cells.

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1. Introduction

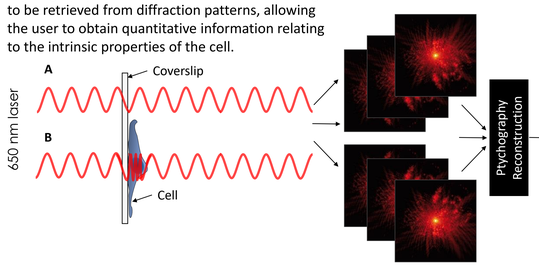
AIM:

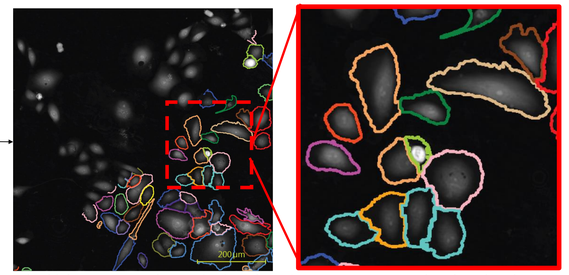
Use of Label-free live cell imaging to perform an advanced scratch wound assay, revealing a morphological and behavioral phenotype for cell metastasis, at a single cell level

- Breast cancer is the most common cancer in women worldwide and in ~30% incidences becomes metastatic and incurable.
- In numerous cancer cell types, the migratory and invasive capabilities required for metastasis have been reported to be linked to the expression of functional voltage-gated sodium channels (VGSCs).
- Although previous reports have highlighted that cancerous cells exhibit a more depolarised resting membrane potential (Vm) than somatic cells, no evidence exists that attributes this phenomenon to the pro-migratory action of VGSCs.
- We utilised Phasefocus™ time-lapse ptychographic microscopy to perform a **Novel and Advanced** scratch wound assay to study the role of VGSC-mediated V_m depolarisation upon the migration of MDA-MB-321 breast cancer cells following treatment with TTX and DrugX.

2. Label-free Quantitative Phase Image Acquisition and Analysis

Ptychography enables the phase information of light to be retrieved from diffraction patterns, allowing the user to obtain quantitative information relating to the intrinsic properties of the cell.





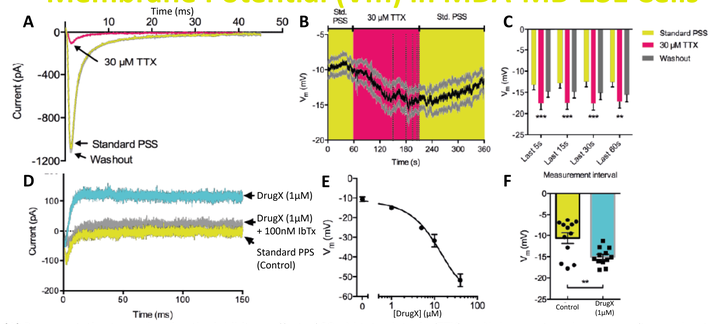
Waves of light that pass through the cells **(B)** are delayed with respect to those that pass through the coverslips only **(A)**. This delay is know as a phase shift. The extent of the phase shift is determined by both the refractive index (RI) and the thickness of the cell. Data collected as diffraction patterns undergo a ptychographic reconstruction to give a **high-contrast** and **quantitative phase image**.

The **high-contrast** and **quantitative phase images** are processed and analysed using the Phasefocus Cell Analysis Toolbox Software™ (CAT), yielding population based metrics, but most importantly **single-cell morphological** and **kinetic phenotypic** data.

Marrison, J *et al.*, 2013. Ptychography-- a label free, high-contrast imaging technique for live cells using quantitative phase information. Sci Rep 3, 2369.

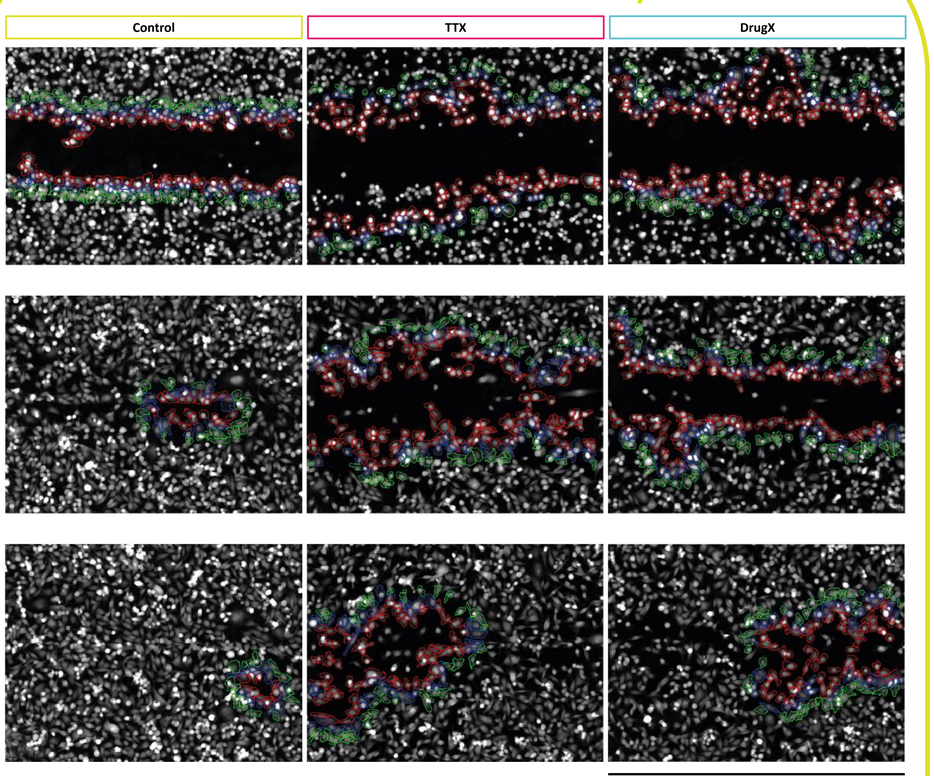
Suman, R *et al.*, 2016. Label-free imaging to study phenotypic behavioural traits of cells in complex co-cultures. Sci Rep 6, 22032.

3. TTX and DrugX Hyperpolarises Membrane Potential (Vm) in MDA-MB-231 Cells



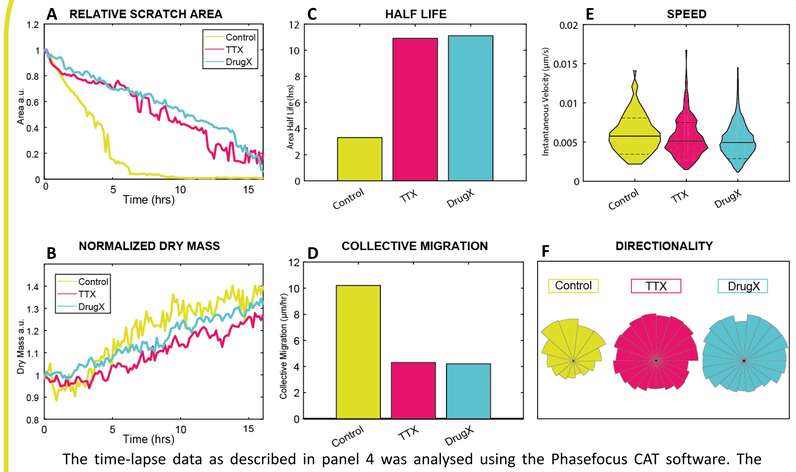
(A) Representative trace showing the inhibitory effect of 30 µM tetrodotoxin (TTX) on Na^+ current, and recovery after washout. The cell was held at -120 mV for 250 ms before depolarising to -10 mV for 50 ms. **(B)** V_m in control, after TTX (30 µM) treatment and following washout. **(C)** Quantification of V_m within the indicated measurement intervals after TTX treatment. **(D)** Representative cell recorded using perforated patch clamp technique showing activation of BK_{Ca} channels outward current using 1 µM DrugX. **(E)** Dose-dependent effect of DrugX on the steady-state V_m **(F)** Effect of DrugX µM) on steady-state V_m of MDA-MB-231 cells.

4. Label-free Quantitative Phase Imaging of a Scratch Wound Closure Assay



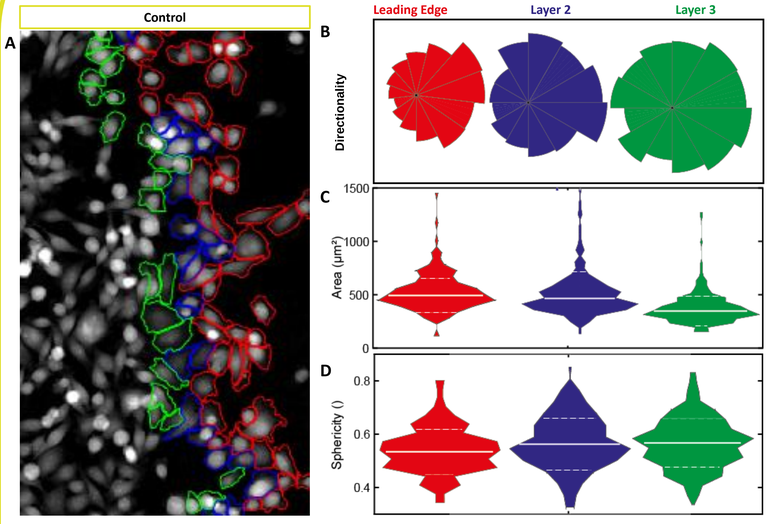
Label-free time-lapse imaging was performed using the Phasefocus microscope to monitor the scratch wound migration process. A 850µm x 650µm label-free, high contrast and quantitative image of the scratch was acquired every 9 minutes for a total period of 16 hours. of MDA-MB-231 cells were with either TTX (30µM) or DrugX (1µM) and compared control. Images were analysed using the Phasefocus Cell Analysis Toolbox (CAT) software. Here both the population based scratch wound area; but more impressively the single cell metrics are calculated.

5. Population and Single Cell Metrics from Scratch Wound Analysis



The time-lapse data as described in panel 4 was analysed using the Phasefocus CAT software. The relative scratch area (compared to t 0) was plotted for each treatment. Both TTX and DrugX reduced the rate of wound closure when compared to control (**A**). This can be seen as an increase in the half life for wound closure and a decrease in collective migration (**C and D**, respectively). To differentiate between migration and proliferation during wound closure, the relative dry mass was measured. There was little difference in the rate of dry mass density between the treatment groups indicating cell proliferation did not impact wound closure (**B**). To overcome any potential influence of cell proliferation when measuring wound closure at the population level, cell migration/motility was measured directly through cell segmentation and tracking of the cells at the leading edge. Here we show that TTX and DrugX reduce both the cell speed but also the directionality (**E and F**).

6. Investigating Propagation of Directionality and Morphology from the Leading Edge Cells



To further demonstrate the advancement of the scratch wound assay enabled by the Phasefocus image acquisition and analysis, the leading edge cells, layer 2 and layer 3 were analysed independently (**A**). Here we show that cells at the leading edge posses a stronger directionality that is perpendicular to the wound when compared to Layers 2 and 3 (**B**). Furthermore, cells show a reduction in the cell area together with an increase in cell sphericity that propagates from the leading edge into the confluent region (**C and D**).

7. Summary

- Label-free Quantitative Phase Imaging enables quantification of a morphological and behavioural phenotype for cell metastasis, at a single cell level (Panel 2)
- Both DrugX and TTX were shown to effectively hyperpolarise the membrane potential of MDA-MB-231 cells through independent mechanisms (Panel 3)
- Time-lapse label-free quantitative phase imaging acquired using the Phasefocus microscope facilitated the image analysis ant both population and single cell level (Panel 4)
- Population level analysis shows that TTX and DrugX cause a reduction in the rate of wound closure without affecting the rate of cell proliferation (Panel 5)
- Single cell analysis at the the leading edge of the scratch gives a direct measure of cell motility and migration. TTX and DrugX reduced both cell directionality and speed (Panel 5)
- The Phasefocus CAT software enables the independent analysis of leading edge cells but also consecutive layers into confluent area, revealing changes in both the behavioural and morphological phenotypes (Panel 6)