Characterisation of the subpopulations of heterogeneous primary prostate epithelial cell cultures derived from patient tissue

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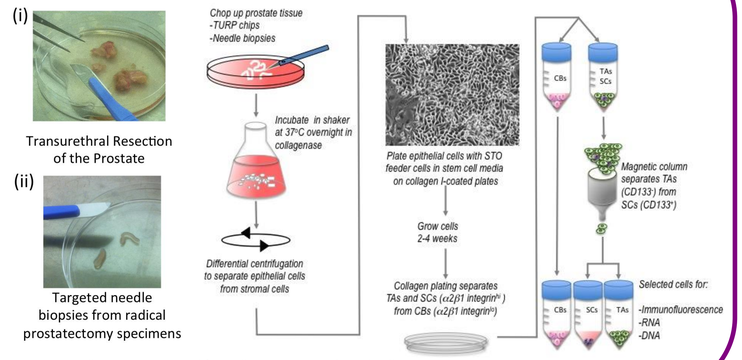
1. Introduction

- **Cell lines do not represent tumour heterogeneity or patient variability**. There is a need for a better model to carry out pre-clinical testing in order to give greater chance of success in clinical trials.
- To address this need, the use of primary cell cultures derived from patient tumours is becoming more desirable and more common.
- **Primary cell cultures represent intra- and inter-patient heterogeneity.**
- In order to develop a successful treatment, all cell types must be targeted, and so combination treatments are likely to be more successful than monotherapies.
- Here, we present the use of ptychography, a label-free imaging technique, to characterise primary prostate epithelial cultures derived from patient tumour tissue.

AIM: Use label-free live cell imaging to characterise primary prostate cell cultures, in order to optimise their use as a pre-clinical testing model.

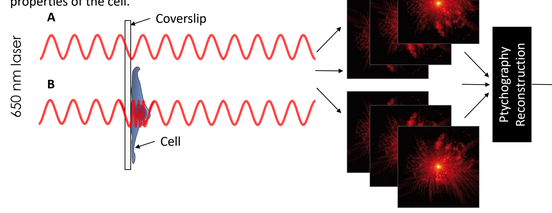
2. Culturing Primary Cells from Prostate Tissue



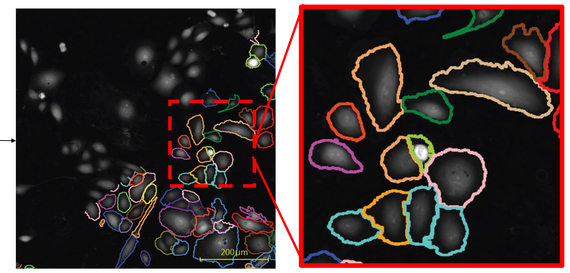
SCs = Stem Cells, TA = transit Amplifier Cells, CB = Comitted Basal Cells

3. Label-free Ptychographic 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.

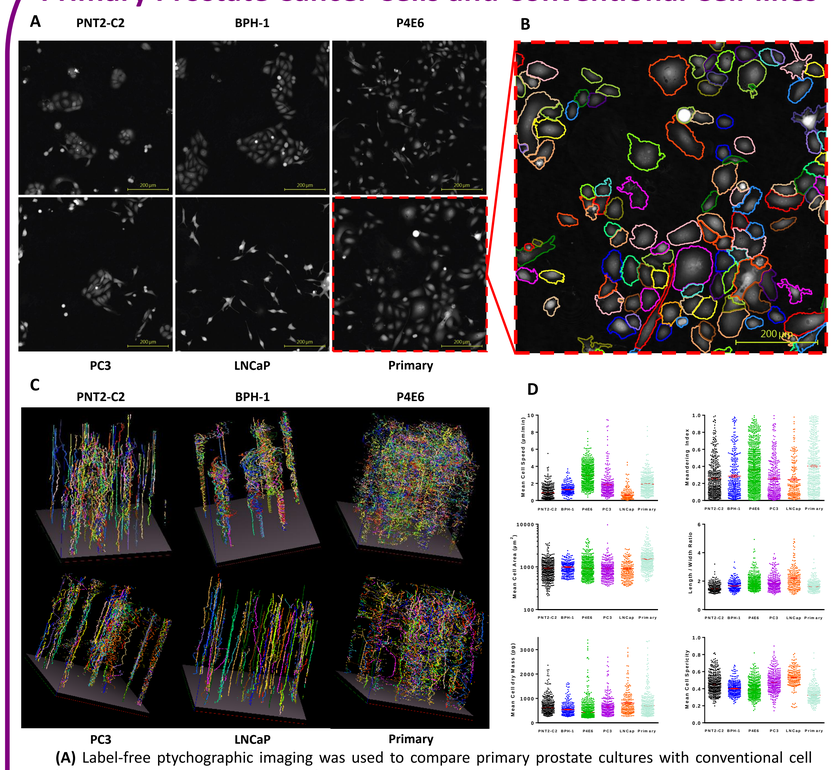


Waves of light that pass through the cells **(B)** are delayed with respect to those that pass through the coverslips only **(A)**. This delay is know as a phaseshift. The extent of the phase shift is determined by both the refractive index (RI) and the thickness of the cell. Data collected as diffraction 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), population based metrics, but most importantly **single-cell morphological** and **kinetic phenotypic** data.

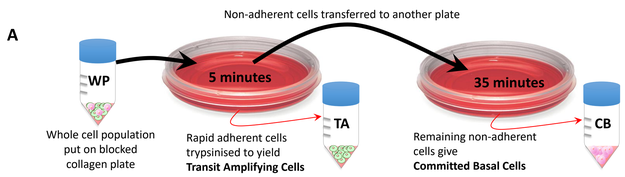
4. Label-free Characterisation of Primary Prostate Cancer Cells and Conventional Cell-lines



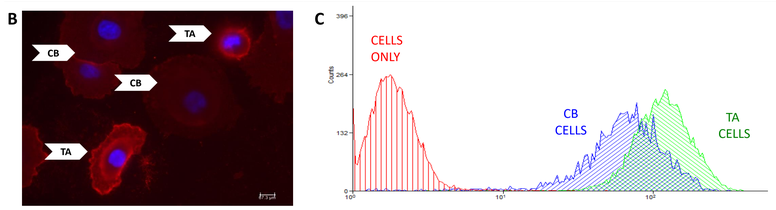
(A) Label-free ptychographic imaging was used to compare primary prostate cultures with conventional cell lines. **(B)** Following segmentation and tracking of individual cells over 72 hours, tracks of individual cells are represented **(C)** and clearly show that P4E6 cells and Primary cells travel a much further distance than the other four cell lines. P4E6 originates from a localised cancer whereas PC3 and LNCaP cells are from a bone and lymph node metastasis. PNT2-C2 originates from normal prostate and BPH-1 from benign prostatic hyperplasia. PC3 and LNCaP are the cells typically used for drug-testing. **(D)** Other parameters tested show a clear difference between primary cells and conventional cell lines, particulary meandering index and cell area.

5. Analysis of Separate Sub-Populations from Primary Prostate Cell Cultures

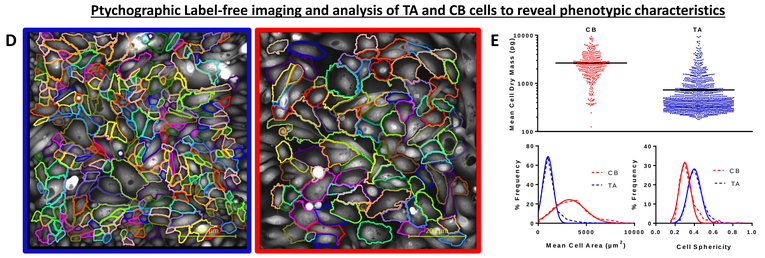
Selection of primary prostate cell subpopulations by collagen adherence



Immunofluorescence and Flow Cytometry analysis of TA and CB subpopulations based on expression of CD49b (α2β1integrin).

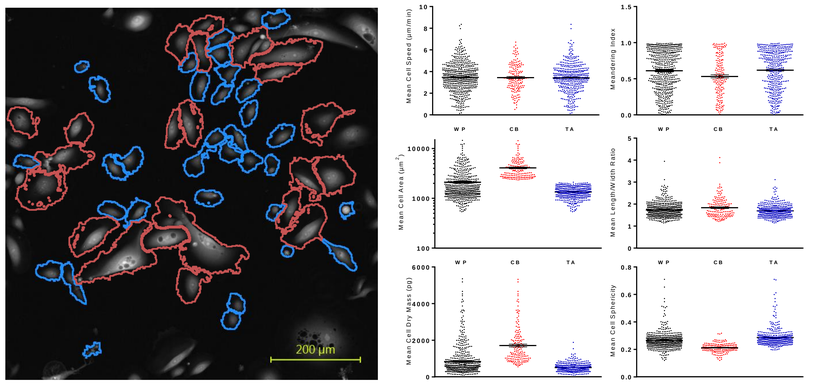


Ptychographic Label-free imaging and analysis of TA and CB cells to reveal phenotypic characteristics



A, Whole cell populations (WP) of primary prostate cancer cell cultures were separated into, Transit Amplifying (TA) and committed Basal (CB) cells based on their collagen adherence properties. TA and CB cell exhibit a subtle difference in α2β1integrin expression **(B)**. However, as demonstrated by flow cytometry **(C)** the difference is not distinct enough to clearly separate the populations. Ptychographic label-free imaging allows for the identification of morphological and kinetic phenotypes at a single cell level. **D,** Label-free timelapse images of TA and CB cell populations were acquired using the Phasefocus Livecyte System. Image analysis performed using the PhaseFocus CAT software, revealed phenotypic differences between the TA and SB populations **(E)**.

6. Label-free single-cell Analysis of Heterogeneous Primary Prostate Cell cultures



Since we were able to identify key differences between the two known subpopulations in primary prostate cultures (TA and CB cells) using label-free imaging, we then tested out those parameters on a heterogeneous (whole population – WP) culture of primary prostate cells. We were successfully able to identify the two subpopulations within the mixed culture. CB cells clearly have a larger cell area, overall bigger dry mass, and slightly reduced cell sphericity. **This shows that cells can be identified by a unique ptychographic signature.**

7. Summary

- Primary prostate cells have very different behaviour and characteristics in comparison to conventional cell lines.
- Analysis of separated populations of TA and CB cells (subpopulations of primary prostate cells) has determined different behaviours and characteristics, and thus each subpopulation has a unique ptychographic signature.
- We can use this unique ptychographic signature to identify the TA and CB subpopulations within a heterogeneous culture of cells.

FUTURE WORK: A full characterisation of all types of patient-derived tumour cells, alongside analysis of their response to current and novel drugs will allow assessment of detailed biological effects of the drugs tested as well as identification of resistant cells. This could lead to patient cells becoming part of the drug development pipeline, which will ultimately result in targeted and patient stratified therapies that take into account intra- and inter-tumour heterogeneity.