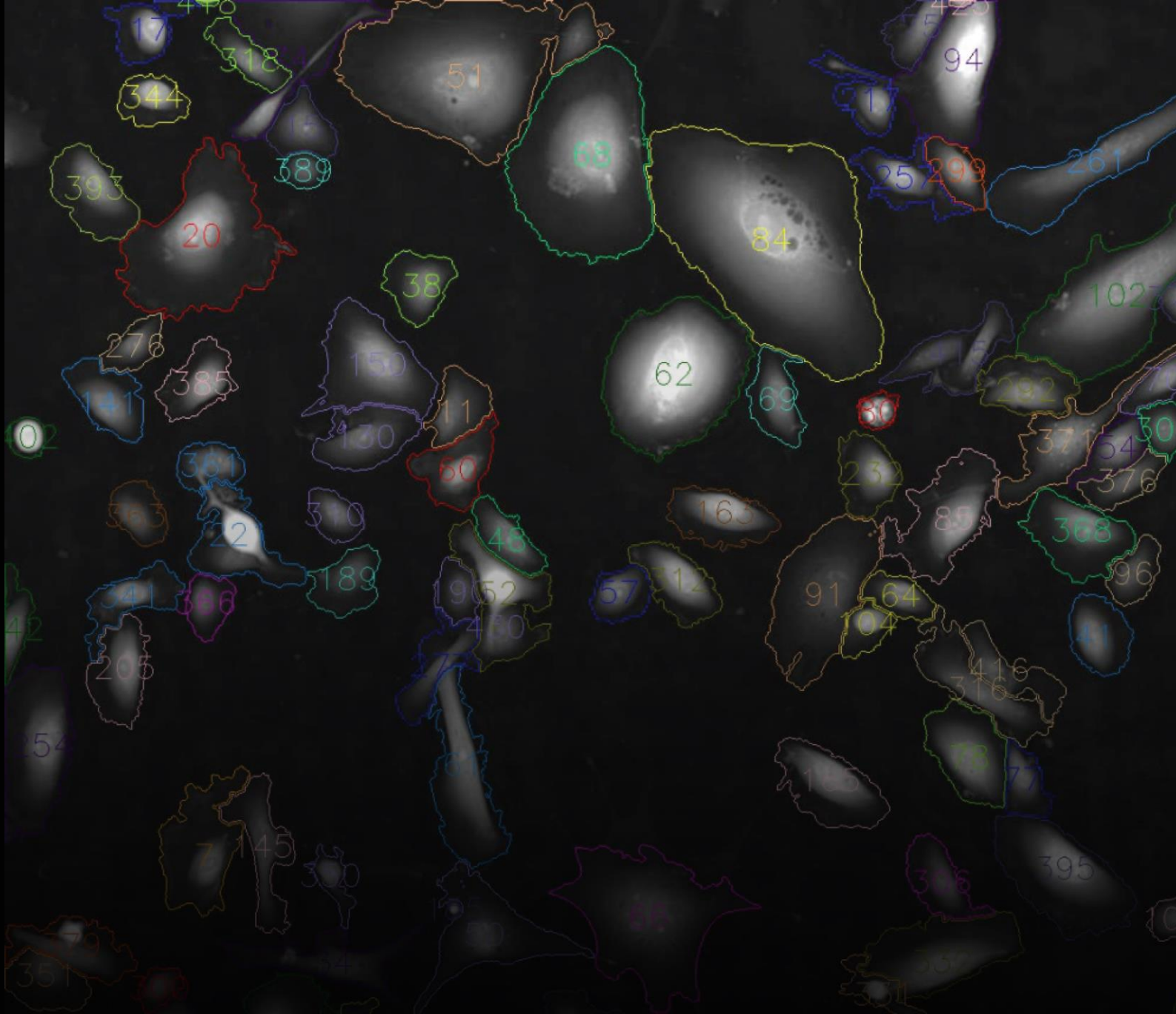


Characterisation of sub-populations of heterogeneous patient-derived primary prostate epithelial cell cultures

Application Note

Analyse and extract parameters to identify heterogeneity within mixed cancer cell populations
Enable investigation of combination therapies on primary cancer cultures

- Heterogeneous primary prostate cancer epithelial cells imaged in a non-perturbing manner
- Extract multi-parametric data to distinguish sub populations within complex culture
- Subpopulations classified and phenotypic fingerprint identified

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Introduction

Patient-derived primary cell cultures are becoming more desirable to carry out preclinical studies, since they provide an improved model for tumour heterogeneity and they represent intra- and inter-patient diversity far more accurately than traditional cell lines. To develop successful treatments, specific sub-populations of cell types must be specifically targeted, and so combination treatments are likely to be more successful than monotherapies. In this application note a non-perturbing imaging modality is utilised to image and characterise heterogeneity within primary prostate epithelial cultures derived from patient tumour tissue.

Methods

Cell populations of primary prostate cancer cell cultures were initially separated into two sub-populations based on their collagen adherence properties - Transit Amplifying (TA) and Committed Basal (CB) cells. The CB and TA cell phenotypes were initially investigated using Immunofluorescence and flow cytometry. The sub-populations were also analysed with the Livecyte's unique label free Quantitative Phase Imaging (QPI) modality and characterised with the Cell Analysis Toolbox (CAT). The unique phase metrics identified from the separated populations were then employed to investigate if the two subpopulations could be successfully identified within a mixed population (WP). The analysis was then extended to this WP culture of primary prostate cells to investigate the ability of the Livecyte system to automatically identify the two subpopulations within the mixed culture.

Results

TA and CB cell exhibit a subtle difference in α2β1 integrin expression as shown in the fluorescence image in Fig 1(a). However, as demonstrated by flow cytometry in Fig 1(b) the difference is not distinct enough to clearly separate the populations.

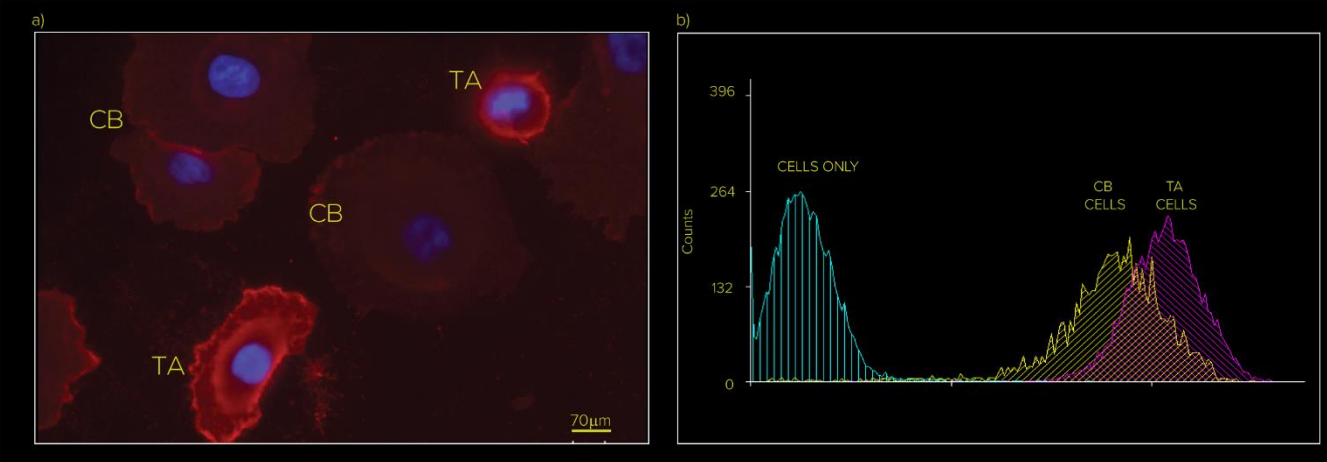


Figure 1– (a) **Immunofluorescence** and (b) **Flow Cytometry analysis** of TA and CB subpopulations based on expression of CD49b (α2β1 integrin)

QPI time lapse was acquired for the TA cells and the CB cells. Single frames for each subpopulation (TA and CB) are presented in Fig. 2. The CAT software was used to segment each individual cell within the time lapse and to extract the metrics displayed in Fig. 2. Individual cell segmentation allows for multi-parametric data to be extracted from a single population. In this case, morphological and dynamic phenotypes for every cell were extracted at every time point.

Fig. 3 displays a single frame from the whole population time lapse and the extracted metrics identifying the two subpopulations within the mixed culture. CB cells clearly have a larger cell area, overall larger dry mass and a slightly reduced cell sphericity. This illustrates how the cells may be classified into WP, CB and TA groups per the distribution of speed, area, dry mass, sphericity, ellipticity, and meandering index.

The segmented images of each population are clearly distinguishable. It is clear both visually and from the data extracted that the CB cells have a much larger area and a lower sphericity. The quantitative nature of the QPI technique enables volumetric data to be extracted. In this case the CB cells clearly demonstrate a higher dry mass. Moreover, there is a greater variance in the cell area of the CB population. The statistics clearly indicate that the single cell analysis agrees with the results from flow cytometry analysis presented in Fig 1b).

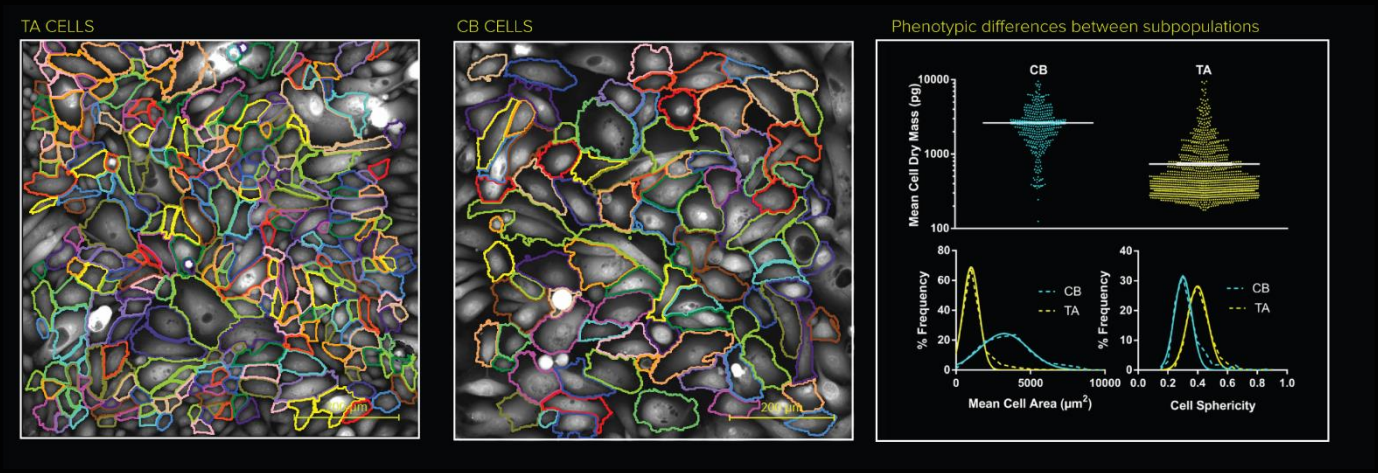


Figure 2 – Individual cell segmentation of the TA and the CB populations. Metrics calculated from the Livecyte's Cell Analysis Toolbox (CAT) relating to each population displays distinct phenotypes.

Fig 2 indicates that it is possible to distinguish key differences between the two known subpopulations in primary prostate cultures (TA and CB cells) with the QPI technique. The subpopulations were combined into a heterogeneous (whole population – WP) culture of primary prostate cells and this WP was then analysed on the system.

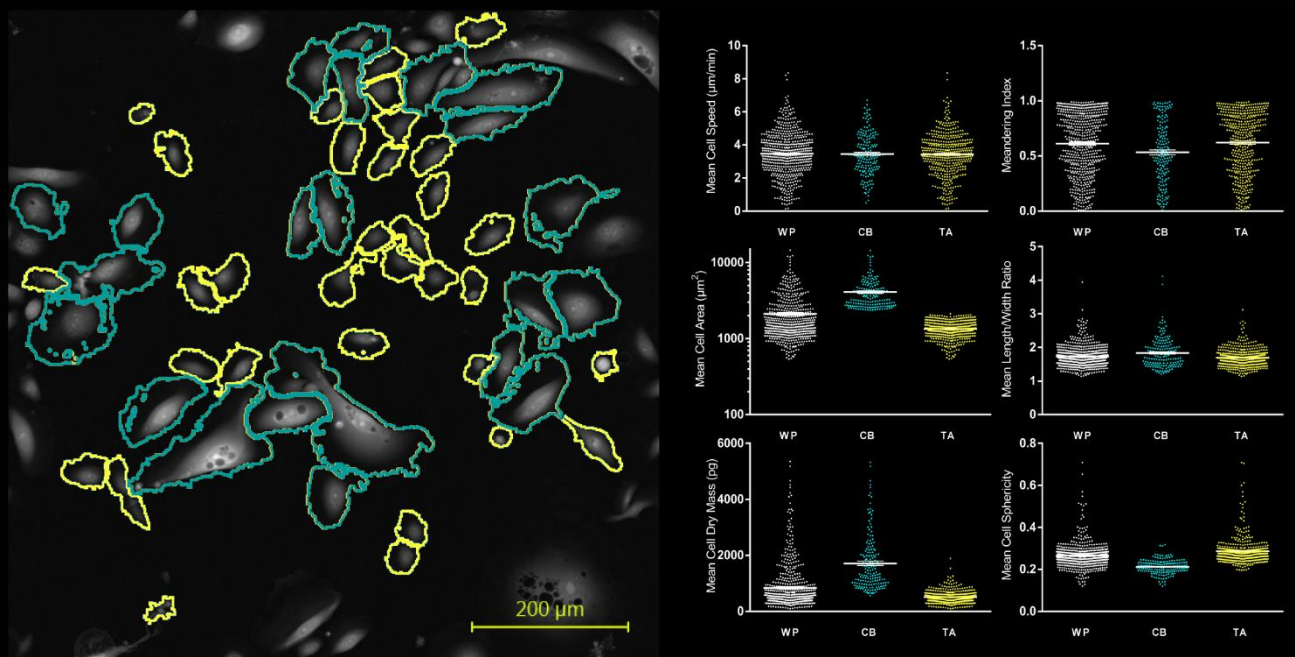


Figure 3 – Individual cell segmentation of the TA and CB subpopulation within a heterogenous culture. The suite of metrics extracted using the Livecyte's Cell Analysis Toolbox (CAT) software illustrate distinct phenotypic differences between the subpopulations.

Conclusion

This application note demonstrates the use of the Livecyte system to both image heterogeneous cell populations and to combine the multiparametric information extracted to classify and distinguish automatically sub-populations within complex co-cultures. The non-perturbing nature of the QPI technique ensures that primary cell cultures can be confidently imaged without any photo-induced behaviour or the uncertainty in cell behaviour from the addition of fluorescent labels. The Livecyte system represents a powerful tool in the development of combination therapy drugs on patient derived heterogeneous cancerous cell cultures.

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