

# Differences in dynamic and morphological phenotypic characteristics enabled by Label-Free individual cell analysis | **Application Note**

## A case study to reveal subtle differences in the behaviour of cell populations

Differences in phenotypic characteristics reveals subtle reaction to treatment

- Reveal differences between treatments that are not resolved by a population averaged approach
- Extract multi-parametric data to reveal a more refined description of population behaviour
- Ensure native cell behaviour is preserved by acquiring data in a non-perturbing manner

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# Introduction

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Segmentation of individual cells within traditional label-free images can be very challenging. The low-contrast and artefact-ridden nature of these images often completely hinders robust individual cell segmentation (e.g. Phase contrast and DIC). Subsequently, researchers resort to a coarser, population averaged approach which can result in more subtle cell behaviour becoming obscured and thus erroneous conclusions can be drawn relating to the true conduct of the population. In addition to unlocking a greater refinement in the cell population detail, individual cell analysis over a time course allows the extraction of dynamic phenotypic description of each individual cell. In this application note a Quantitative Phase Imaging (QPI) technique is utilised to produce time course data for 3 treatments. The subsequent data is analysed to an individual cell level to reveal distinct differences in the cell behaviour which would not be distinguished in a population averaged analysis.

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# Methods

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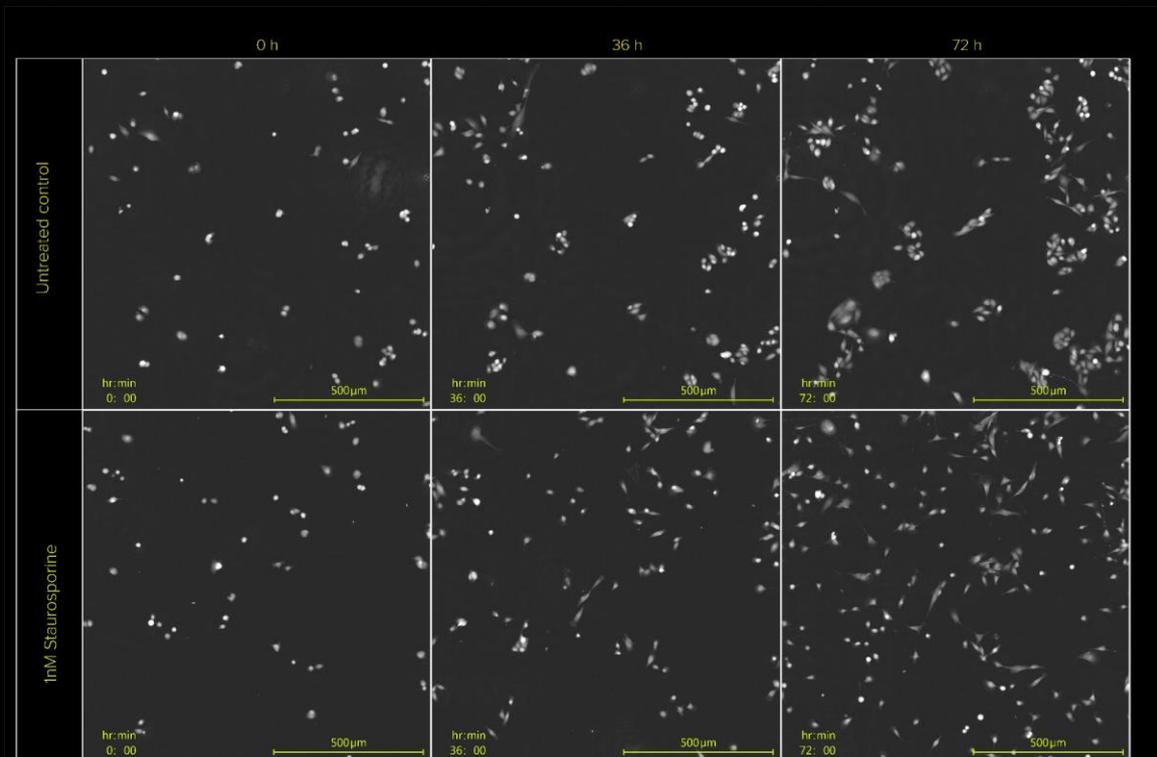
MDA-MB-231 cells treated with 1 nM and 10  $\mu$ M of Staurosporine. The Livecyte system was utilised to obtain label free time course (10X/0.25, 72h, 10min imaging interval) images of the two treated populations and a control population. The Livecyte Cell Analysis Toolbox (CAT) was employed to extract data resolved to both population averaged and an individual cell level. For comparison, the populations were also measured using trypan blue exclusion on the Vi-Cell from Beckman Coulter.

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# Results

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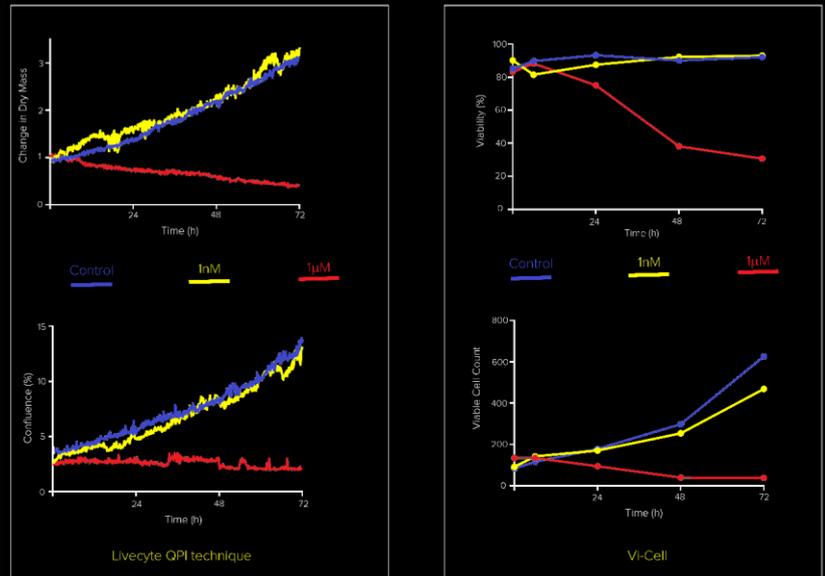
Fig. 1 displays example frames from the Livecyte QPI modality. This shows extracted frames for both the control and the 1 nM Staurosporine treatment. Due to the low dosage of Staurosporine there is very little visual evidence of any increase in



**Figure 1— Example QPI images of control and 1nM Staurosporine.** Individual frames extracted at 0h, 36h and 72h indicating no discernable increase in cell death between both populations.

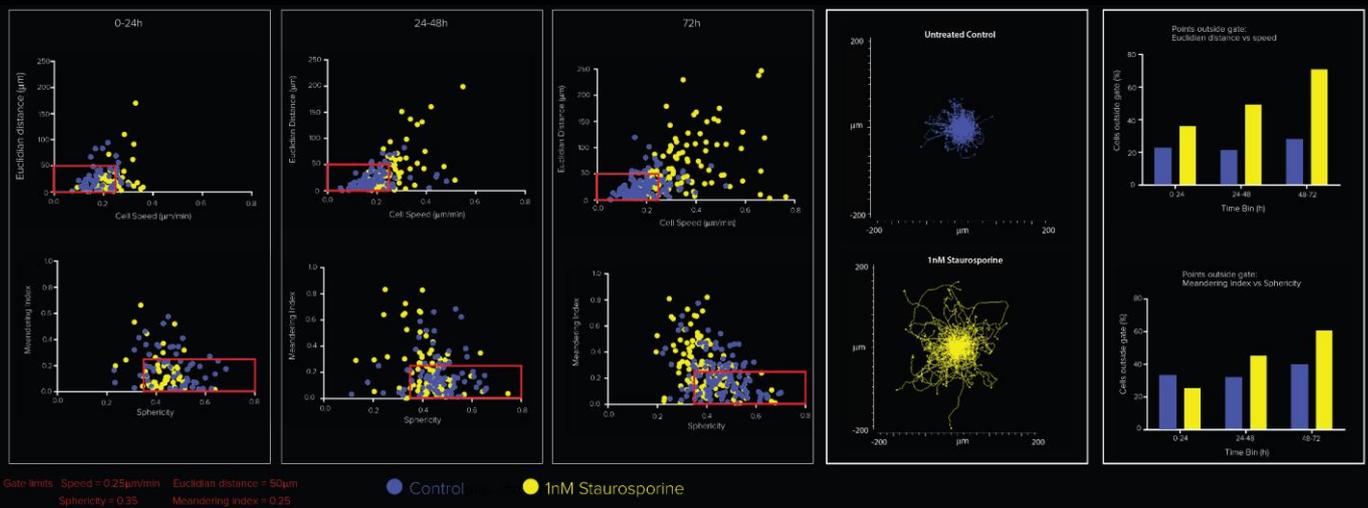
cell death. Fig. 2 displays a population averaged result from both the Livecyte and the Vi-Cell. The Livecyte (left hand column) indicates a similar increase in both dry mass and confluence for both the control and 1 nM treatment. Conversely,

for the larger dosage of Staurosporine, cell death is indicated by the decrease in both the population metrics. The trend was validated by another population based technique (Vi-Cell). In this case, both population indicators (cell viability and cell count) suggest a distinct difference between the 10  $\mu\text{M}$  dosage and that of the control and 1 nM dosage. Again, like the Livecyte population approach there is no distinguishable difference for cell viability. There is a subtle change in cell viability count at 72 hours, which would warrant further investigation to determine its provenance. However, no definitive conclusions can be drawn at this point. With the Livecyte system we can investigate further, as the cell population is not destroyed to extract the information and the further refinement in analysis required is achieved with individual cell segmentation.



**Figure 2 – Population level analysis for both the Livecyte (continuous) and the Vi-Cell (fixed time points).** Both systems indicate no obvious differences between the control and low dose of Staurosporine.

Fig.3 displays the results achieved enabled by individual cell segmentation. Livecyte CAT software was used to segment each individual cell in all 3 populations and compare its dynamic and morphological phenotypes. The automated segmentation from CAT and post filtering (to exclude events where cells  $<200\mu\text{m}^2$  in area and tracked for less than 6.7 h) reveals the subtle differences in cell behaviour between the two populations. Fig. 3 displays the differences in the dispersal of individual cells between the untreated control population and the low dose 1nM treatment.



**Figure 3 – Individual cell segmentation of the control population and the 1nM treatment.** Morphological and Dynamic phenotypic analysis of populations combine to reveal differences between populations indicating that sub-toxic levels of Staurosporine do elicit a response in cell behaviour.

Combining all the data displayed in Fig.3, it can be concluded that 1 nM Staurosporine caused a subset of cells to exhibit more directed motion, become elongated, and roam further from neighbouring cells when compared to control cells. The results suggest treatment of MDA-MB-231 cells with sub-toxic concentrations of Staurosporine elicits a pro-migratory phenotype in a subset of cells.

Revealing such motility information is essential in many aspects of biology, e.g. immune regulation, tissue regeneration and embryogenesis. Deregulation of cell motility can result in diseases such as cancer, autoimmune disorders, neurological diseases and chronic inflammation.

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## Conclusion

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This application note demonstrated how individual cell segmentation can reveal the subtle differences in dynamic phenotypic behaviour and morphological phenotypic characteristics between treated populations. Population level indicators of cell growth, confluence, proliferation and viability can only conclude that cells were only killed with 10  $\mu$ M Staurosporine. Individual cell data revealed that the subtle effect caused by the 1 nM Staurosporine dosage was only revealed when the behaviour can be refined to an individual cell level.

This particular case study demonstrates that by using the non-perturbing acquisition modality of Livecyte, in combination with the power of individual cell measurements, dynamic and morphological phenotypes can be employed to annotate the effects of drugs/treatments that are not captured by population-level approaches nor endpoint analysis.



For more information on the benefits of the Livecyte system, to access application notes and for additional product information, please visit:

[www.phasefocus.com/livecyte](http://www.phasefocus.com/livecyte)

A sample of time-lapse videos can be found at:

[www.youtube.com/phasefocuslimited](http://www.youtube.com/phasefocuslimited)

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