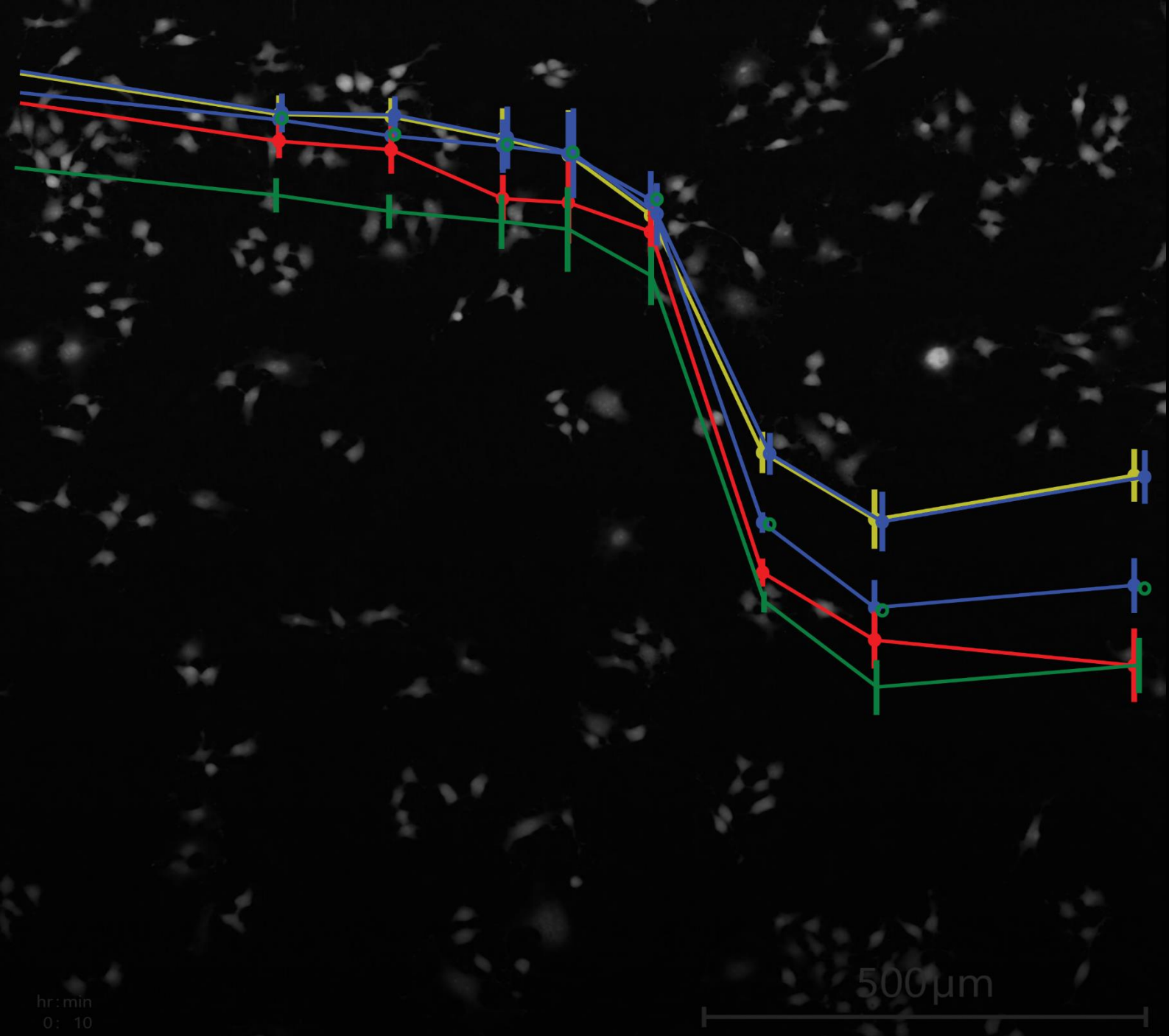

Label free calculation of cell death dose response curve | Application Note

Label Free dose response

Produce a Staurosporine dose response curve without the use of fluorescent labels

- Produce dose response curves without the use of fluorescent labels
- Perform assay on a 96 well plate to enable repeats and variation in treatments
- Utilise dry mass to indicate cell death
- Utilise correlative fluorescence capabilities to validate measurement in specific wells

phasefocus

Introduction

The dose response curve refers to the relationship between a specific biological effect and the concentration of an administrated drug. In this application note we present a dynamic and label-free dose response assay for the death of U251 Glioblastoma cells to a varying dose of Staurosporine. The Livecyte system utilises a Quantitative Phase Imaging (QPI) modality to measure the dry mass of cells over time which can be used to characterise cell death. The ability to perform correlative fluorescence measurements on Livecyte was employed to measure the accumulation of propidium iodide (PI), which is a more conventional measure of cell death. This correlation enables the ability to investigate and validate the use of cellular dry mass as an indicator of cell death.

Methods

U251 Glioblastoma cells were seeded in the central 60 wells of a 96 well plate. A control and nine varying concentrations of staurosporine (with 6 repeats for each) were added across the plate. PI was also added to achieve a correlative fluorescence measurement and to further examine the relationship between dry mass and cell death.

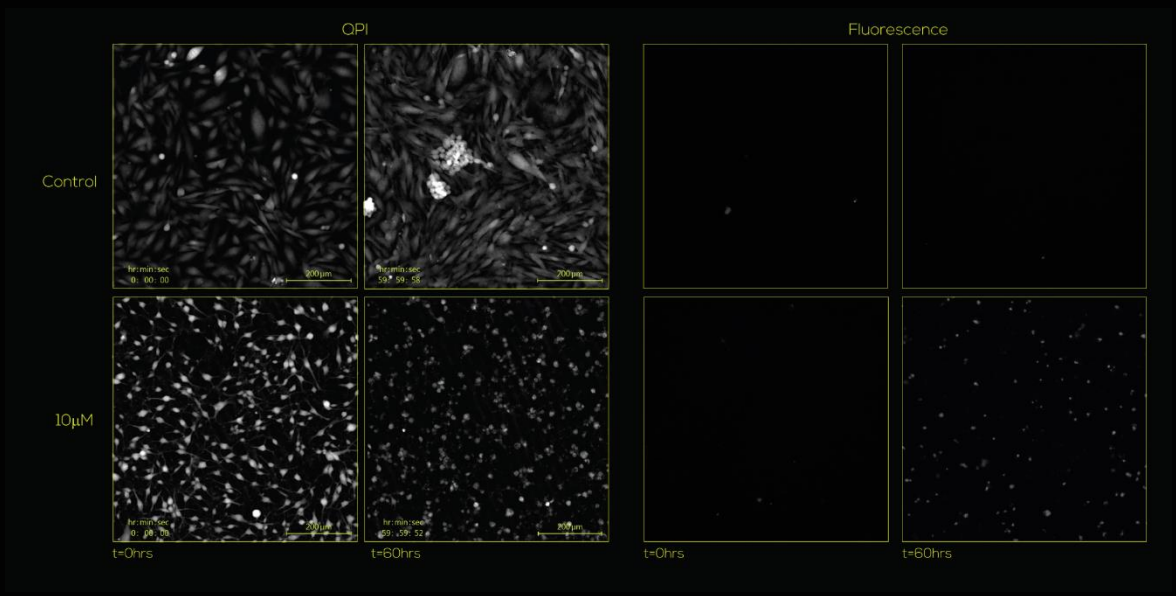

Figure 1 – Example QPI images and fluorescence images for control and treated wells at times 0 hours and 60 hours.

Fig. 1 displays the phase images and equivalent fluorescence images for the control and the 10μM dosage for the start and end of the time course (T = 0 hours and T = 60 hours). Livecyte Cell Analysis Toolbox (CAT) was used to analyse the acquired images and extract the dry mass of the cells in every well.

Results

Fig. 2 displays the changes in relative dry mass over the 60-hour time-lapse for the U251 glioblastoma cells in the absence (Control) and with increasing concentrations of staurosporine (n=6 for each treatment). The relative dry mass is the total mass (as calculated in CAT) of the cell population (which contains approximately 2700 cells at each drug concentration) relative to the initial dry mass (approximately 2 hours after application of the drug). Under control conditions, cells continue to grow and proliferate as expected. This is reflected by an increase in relative dry mass in the first 24-hour period.

After 24 hours the rate of dry mass accumulation is attenuated as the cells reach maximal confluency. At intermediate concentrations (0.01 -0.5μM), growth and proliferation are inhibited by the drug indicating a cytostatic response. For the highest concentrations (1μM, 2μM and 10μM), the cellular mass decays to a minimum after 30-40 hours. This is the point at which all cells in the population have undergone cell death and just residual cellular fragments remain.

Fig. 3a displays the dose response of the cellular dry mass metric. The dose response (relative change in dry mass) is plotted as a function of staurosporine concentration (μM) for several time points (24, 36 and 60 hours). The data exhibits a sigmoidal response function from which the Hall co-efficient and concentration for 50% maximal response (inflection) may be extracted from a mathematical fit. The response amplitude and mid-point increase over time. Figure 3b displays the temporal dependence of the dose response inflection point from fits to the sigmoidal curves at all timepoints. At 24 hours, the concentration for 50% response is found to be 0.56 μM.

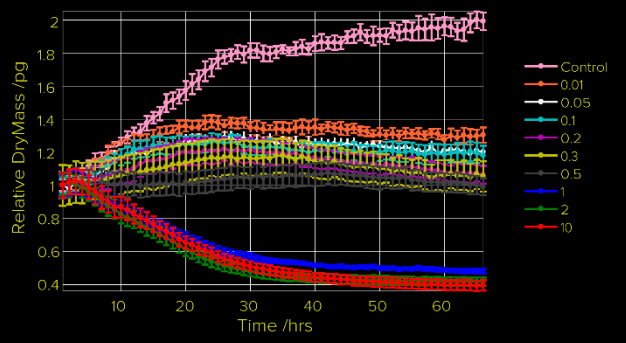

Figure 2 - Dynamic dry mass of the population relative to starting mass as a function of staurosporine concentration in μM. Error bars are standard error.

Fig. 3a

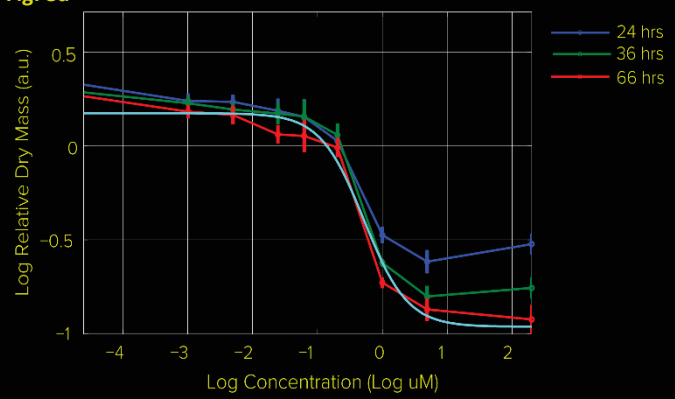

Fig. 3b

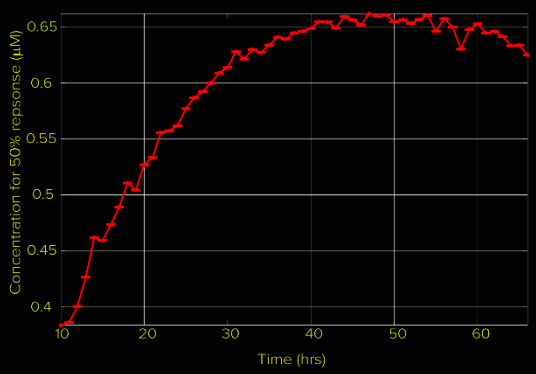

Figure 3 – Relative dry mass dose response. a) The relative mass (log) of the cell population is plotted as a function of (log) drug concentration for all times (1 to 60 hours). Highlighted are three timepoints (green, blue, red lines) to illustrate the sigmoidal function at 24, 36 and 60 hours respectively. The cyan curve is an example fit to the data from 60 hours. Error bars are standard error. b) **Dynamic dry mass dose response.** The does response (concentration for 50% mass loss) plotted as a function of time.

The correlative fluorescence feature of Livecyte was utilised to investigate the relationship between the fluorescence (PI expression) and the QPI images (dry mass metric). Fig. 1 illustrates start-point and end-point QPI and fluorescence images for two wells: control and 10 μM concentration. In the fluorescence case, the start-point of both the control and treated wells contain little PI signal, indicating that few cells have died. At the end-point (60 hours), the PI accumulation is significantly higher for the treated well, indicated an increase in cell death. In the QPI control case, the cells have proliferated and grown, resulting in an increase in total dry mass. For the 10 μM concentration end-point, the majority of the cells have died, reducing the overall decrease in relative dry mass. Fig. 4 illustrates the inverse correlation between the conventional fluorescence based assay (PI accumulation) and the label-free based assay presented.

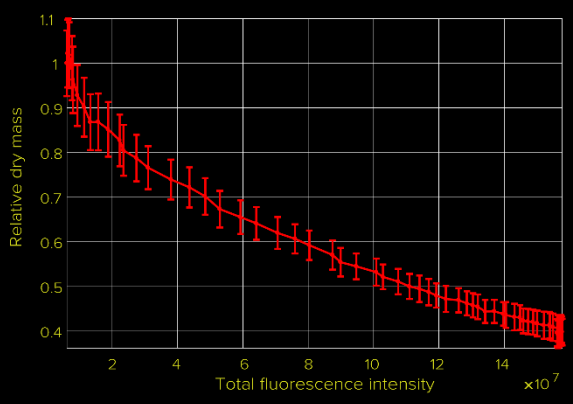

Figure 4 cell mass and fluorescence correlation. The relative inverse mass is plotted as a function of the total fluorescence signal showing a clear correlation between the two data types.

Conclusion

In this application note it was demonstrated that the Livecyte system can be used to produce a Staurosporine dose response curve. The QPI modality enables quantitative assessment of the cell population and can therefore be utilised to define a multitude of cellular activity which would traditionally require labels – in this case cell death. Further to this, the QPI technique enables multi-parametric phenotypic information to be extracted simultaneously from the same population (motility, area, mitotic events etc) so an array of dose response curves can be calculated label free.

livecyte

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

A sample of time-lapse videos can be found at:
www.youtube.com/phasefocuslimited

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