The Best of Both Worlds: Combining Label Free and Fluorescence Imaging with Livecyte

Peter Djali[1], Dr Meetal Jotangia[1]
[1]Phase Focus Ltd, Sheffield, United Kingdom

Fluorescent imaging can reveal much about cellular function but can also be a double-edged sword with both phototoxic and cytotoxic consequences that can impair normal cell function or even induce cell death. Livecyte's label-free live-cell imaging is a powerful quantitative phase technique, generating high-contrast, fluorescence-like images using low powered illumination in which cells appear as bright objects on a dark background. The enhanced contrast increases the robustness of single-cell segmentation and tracking without the need for dyes, maintaining true physiological conditions over a long period of time. Additionally, Livecyte offers the best of both worlds: - intermittent fluorescence imaging reduces the potential detrimental effects and can be linked to the more frequent non-perturbing label-free images.

Our previous studies have reported the detrimental effects to normal cell division of the nuclear live-cell imaging stain SiR-DNA when compared with non-perturbing label free imaging on the Livecyte. The inclusion of SiR-DNA reduced both cell count and total dry mass in a LED power dose-dependent manner relative to non-illuminated and unlabelled conditions suggesting fluorescence altered cells natural growth and proliferation rate. Alongside this, SiR-DNA and illumination increased median cell area which correlated with changes in average dry mass per cell compared to controls. Being able to look at multiple parameters indicates a cell profile of phototoxicity with slowed division and accumulation of mass leading to a large, oversized phenotype.

Such effects will be even more enhanced when dealing with sensitive primary cells such as T-Cells and Neuronal cells. We now show how we can track neuronal outgrowth completely label free, by a unique combination of brightfield and Livecyte label free technique, and how an advanced T-Cell assay can be built in which the sensitive primary effector cells remain entirely label free.

T cell killing assays traditionally yield little or no information on the T to Target interactions and would rely on fluorescence labelling of photo vulnerable primary T cells. However, Livecyte can gain a plethora of T cell interaction kinetics with target cells including the time and number of T cell contacts with a target cell before cell death occurred. All of this without having to label T cells giving more meaningful insights on how your engineered T cells are behaving and more predictive information for in vivo studies.

Livecyte's label free imaging and analysis has also set a new precedence for neurite outgrowth assays. Neuronal cells are also more at risk of phototoxicity limiting our ability to image over long periods of time and to look at network formation dynamically. With Livecyte's advanced analysis recipe, neurite outgrowth can be quantified from the initial changes in cell morphology and migration as cells form attachments through to network formation and metrics such as the number of branching points and total neurite length, gaining a novel understanding of the effect of therapeutic drugs on the dynamics of neurite outgrowth.

There is mounting evidence revealing multiple off-target effects and phototoxicity caused by fluorescence excitation probes with reactive oxygen species being the predominant cause of phototoxicity [1,2]. Livecyte versatility enables either non-invasive QPI only or with a possibility of intermittent fluorescence imaging producing a plethora of time sensitive single-cell information where there is a strong need to protect the cells. This provides invaluable

data to researchers using fluorescence to investigate subtle changes in cell behaviour and phenotype.

References
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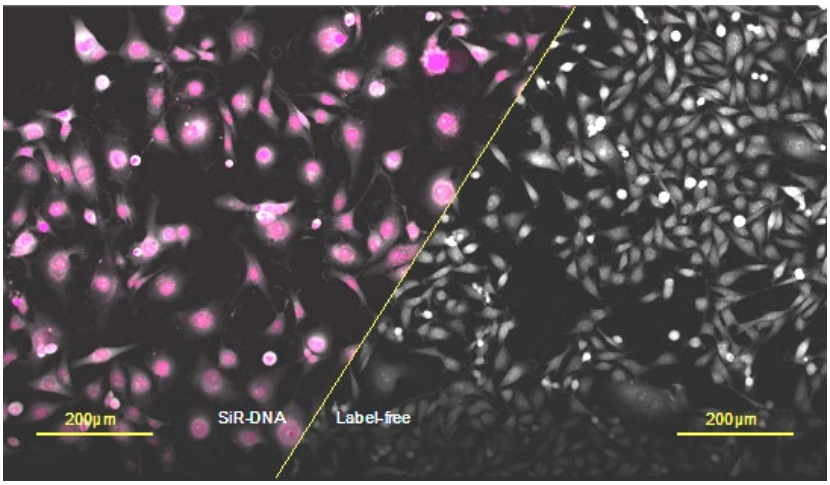
Graphic:



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