Illuminating the truth behind fluorescent labels: The unfriendly side of “live-cell friendly” probes

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Abstract

Ever considered how fluorescent labels could be impacting your experiment? Often overlooked, fluorescence can be a hidden detriment to your cells, at best impairing normal cell function and at worst inducing cell death. However, assessing their long-term impact on cell behaviour can be challenging. Livecyte’s label-free live-cell imaging is a powerful 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. Coupled with this, Livecyte’s correlative fluorescence capability enables fluorescence signal to be captured and linked over time to frequent quantitative phase imaging which can aid in detecting subtle changes in a cell’s response to fluorescence illumination.

To put this to the test we quantified how the nuclear live-cell imaging stain SiR-DNA affected cell phenotype over time using the Livecyte Kinetic Cytometer and Analyse software. MDA-MB-231 cells were treated with either media alone or 250nM SiR-DNA and exposed to a range of LED illumination powers. High contrast images were taken every 20 mins for 120 hours.

The high-contrast label-free images generated by Livecyte enabled us to segment and track cells providing a suite of growth, proliferation and morphology metrics including cell count, total dry mass, cell area and cell dry mass. All this information is conveniently portrayed on the Livecyte Analyse dashboards allowing you to easily correlate and compare treatment changes. 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 creates a cell profile of photoxicity with slowed division and accumulation of mass leading to a large, oversized phenotype.

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] However, the long-term effects of these labels have been relatively unexplored. Livecyte enables extended live-cell imaging producing a plethora of time sensitive single-cell information. The results here highlight the great caution that should be taken when using live-cell friendly probes as cells treated with SiR-DNA and exposed to common fluorescence illumination levels exhibit different phenotypic behaviour to unlabelled cells, even within 24 hours. This provides invaluable data to researchers using fluorescence to investigate subtle changes in cell behaviour and phenotype. Livecyte also gives the added advantage of being able to correlate easily selected treatments versus fluorescence controls. In addition, we understand Fluorescence is needed in many applications which is why Livecyte’s correlative fluorescence capability enables fluorescence signal to be captured infrequently, but the signal from individual cells to be linked over time; long-term fluorescence intensity whilst substantially reducing phototoxicity effects.

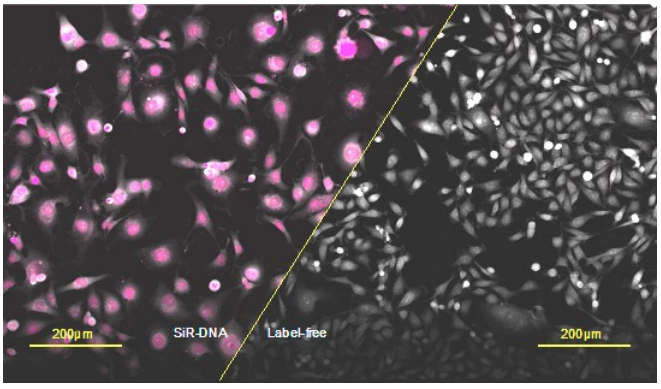

Figure 1: An illustration depicting label-free imaging versus SiR-DNA fluorescence. MDA-MB-231 cells were exposed to 250nM SiR-DNA at 400 µW/mm2 for 120 hours. Fluorescent treated MDA-MB-231 cells appear to be larger with a less characteristic epithelial phenotype compared to unlabelled cells.

References

1. Magidson V, Khodjakov A (2013). Circumventing photodamage in live-cell microscopy. Methods Cell Biol. 114:545-60.

2. Douthwright S, Sluder G 2017. Live Cell Imaging: Assessing the Phototoxicity of 488 and 546 nm Light and Methods to Alleviate it. J Cell Physiol 232(9):2461-2468