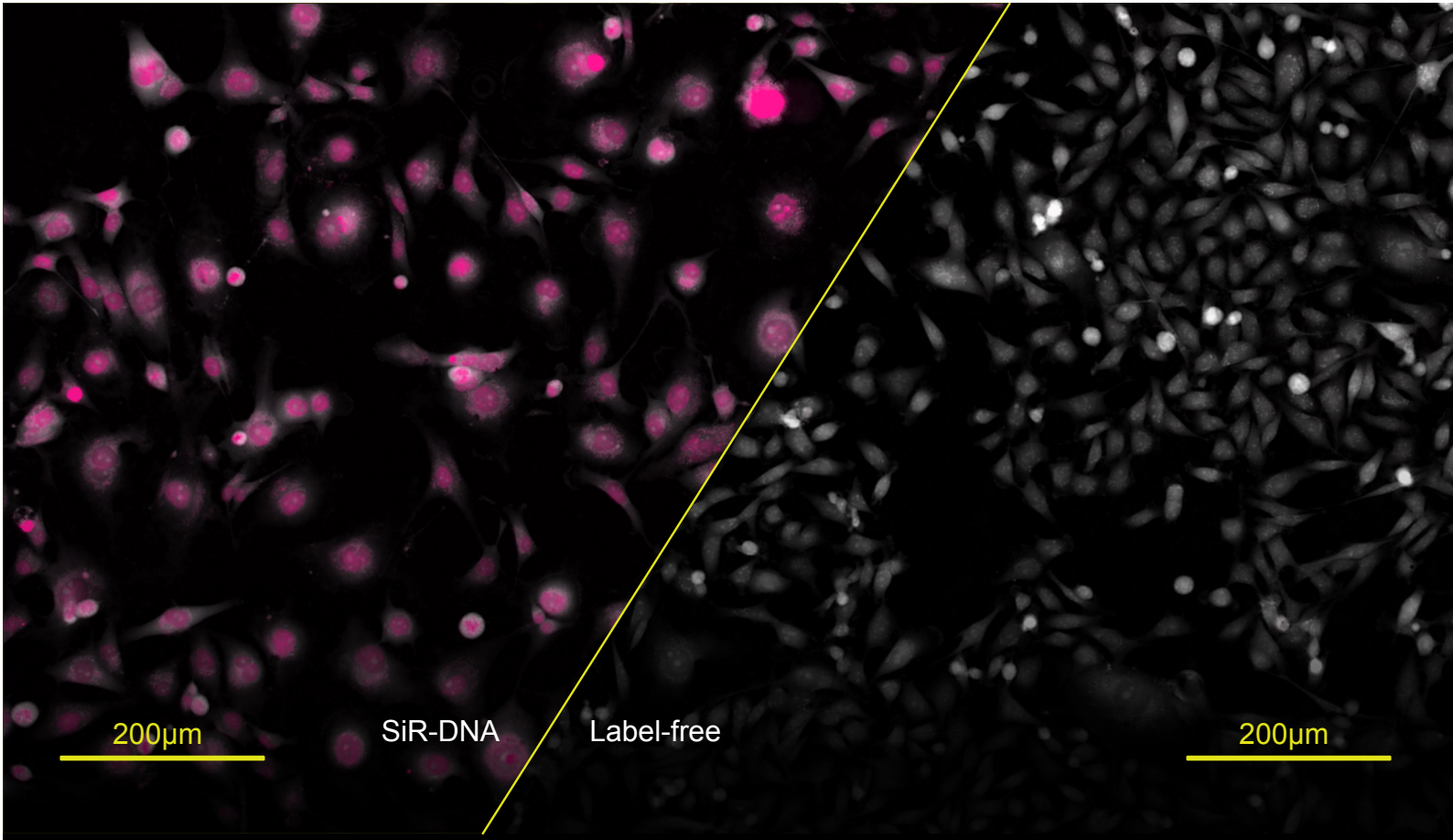


Measurement of SiR-DNA phototoxicity | Application Note

- Far-red DNA label, SiR-DNA, designed for live cell imaging, affects the growth of cells even within 24 hours
- Cells labelled with SiR-DNA and exposed to fluorescence illumination become larger than unlabelled cells
- The label-free single-cell growth metrics produced by Phasefocus Livecyte can be used to directly characterise cytotoxic effects

Application Note AN012 - May 2019

phasefocus

Introduction

Live-cell time-lapse microscopy is an established and powerful technique for the study of mammalian cell biology *in vitro*. Common time-lapse applications use label-free and/or fluorescence approaches, with latter requiring the introduction of dyes or labels.

Labels have the potential to alter normal cell function and induce toxicity [1,2] whilst phototoxicity presents an additional barrier to long-term imaging since the high intensity light required to excite fluorophores can also alter cell behaviour and induce cell death [3].

Reactive oxygen species are the predominant cause of phototoxicity [3]. Cell death is the most apparent outcome of photodamage [4], however subtle changes in cell morphology, motility and proliferation that can have unpredictable effects on experimental outcomes are often overlooked [5].

Phasefocus Livecyte uses ptychography to generate high-contrast, fluorescent-like images, using low powered illumination (4-7μW/mm^2), in which cells appear as bright objects on a dark background. The enhanced contrast in combination with phase retrieval data increases the robustness of single cell segmentation and tracking algorithms without the need for dyes or probes [5]. Ptychography is a form of Quantitative Phase Imaging (QPI); an emerging imaging technique that retrieves phase-delay of light passing through a cell.

This study investigated the effects of far-red fluorescent labelling and LED irradiation on cell behaviour during long-term time-lapse microscopy using a Livecyte Kinetic Cytometer.

Cell Culture

MDA-MB-231 cells were routinely maintained in DMEM + 10% FBS culture medium (DCM) at 37°C with 5% CO_2/95% humidity prior to experiments. Cells were harvested using standard techniques and cell count and viability determined by trypan blue exclusion (ViCell; Beckman Coulter®). Cells were seeded into 30 wells of a 96-well plate at 1600 cells/well and cultured overnight. Media was replaced with either DCM or DCM containing 250 nM SiR-DNA (15 wells per condition) and incubated for 30 minutes prior to imaging.

Resources

- MDA-MB-231 cell line (ATCC® HTB-26™)
- SiR-DNA (Spirochrome SC007)
- DMEM + 10% FBS (v/v; Gibco®)
- 96-well culture plate (Corning® 3603)
- Livecyte Kinetic Cytometer (Phasefocus)
- Livecyte Analyse software (Phasefocus)

Time-Lapse Imaging

High-contrast label-free images were automatically spatially and temporally correlated with fluorescence images using a Livecyte Kinetic Cytometer. Cells were imaged with an Olympus PLN 10X (0.25NA) objective and 1 mm x 1 mm scan region (FOV) per well for 120 hours at 20-minute intervals. Cells were maintained inside an environmental chamber at 37°C with 5% CO2/95% humidity. To determine the effects of irradiation on cell behaviour, cells were exposed to 650nm LED irradiation, in triplicate, via a traditional epi-fluorescence pathway at 0, 80, 200, 300 and 400 μW/mm^2 for 500 ms.

© Phase Focus Limited. AN012 | May 2019

phasefocus

Analysis

Image analysis and automated cell segmentation were conducted by Livecyte Analyse software. Single-cell metrics were produced using the in-built Proliferation and Morphology dashboards. Outputs were formatted for this application note using GraphPad Prism 8 (GraphPad Software).

Results

The effects of LED irradiation on MDA-MB-231 cell behaviour with and without the inclusion of the far-red SiR-DNA stain was assessed using the Livecyte Kinetic Cytometer.

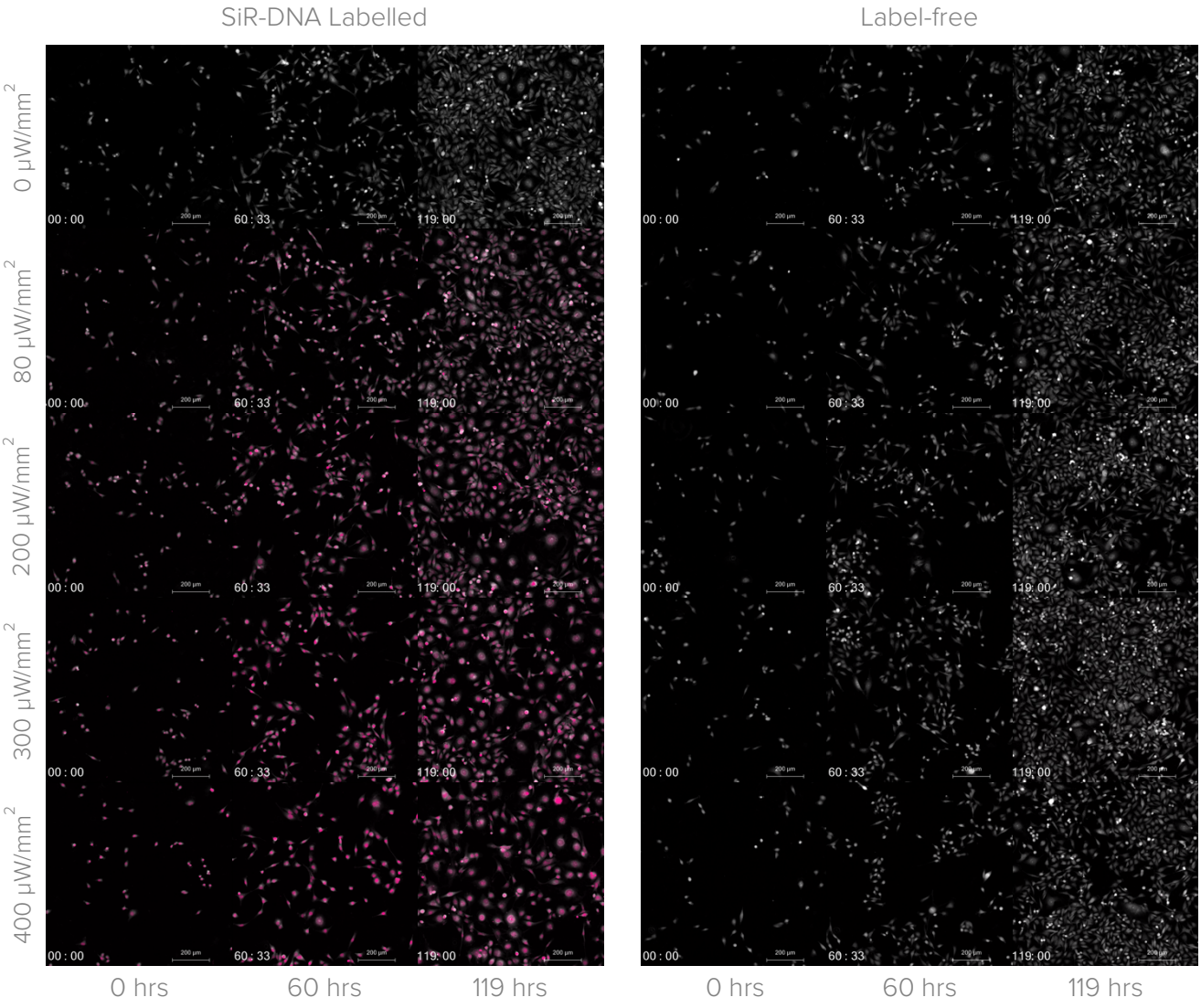


Figure 1: Illustrative QPI and correlative fluorescence images of MDA-MB-231 cells labelled with SiR-DNA (left) and unlabelled (right) at 0, 60 and 119 hours. Irradiation dosage 0-400 uW/mm^2 and scale bar = 200µm.

© Phase Focus Limited. AN012 | May 2019

phasefocus

Cell Proliferation

Irradiation alone did not affect cell proliferation, with similar relative cell counts observed across all doses at each time-point over 120 hours (Figure 2). In contrast, the inclusion of SiR-DNA reduced cell proliferation in an irradiation-dose-dependent manner relative to non-irradiated and unlabelled conditions. Changes in proliferation were observed within 24 hours and became more pronounced over time (Figure 2).

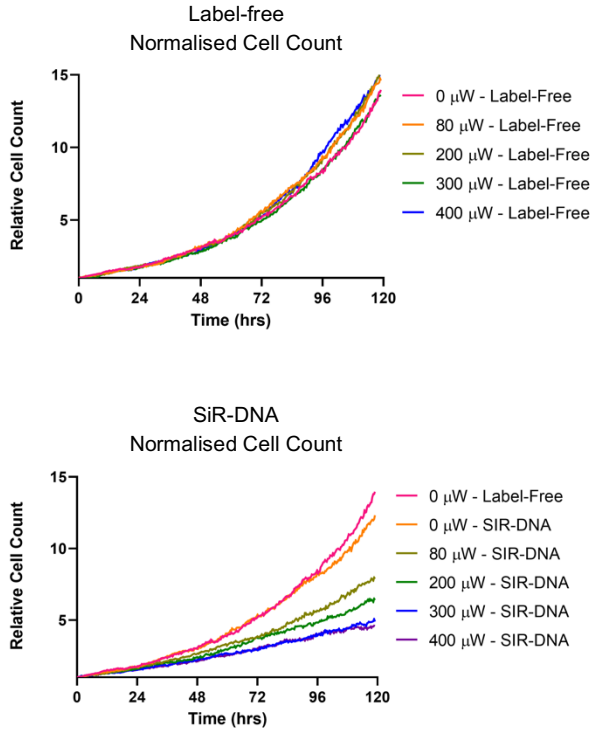


Figure 2: Relative cell count over time for unlabelled (top) and SiR-DNA labelled (bottom) cells at varying irradiation doses. SiR-DNA labelling combined with irradiation inhibits cell proliferation.

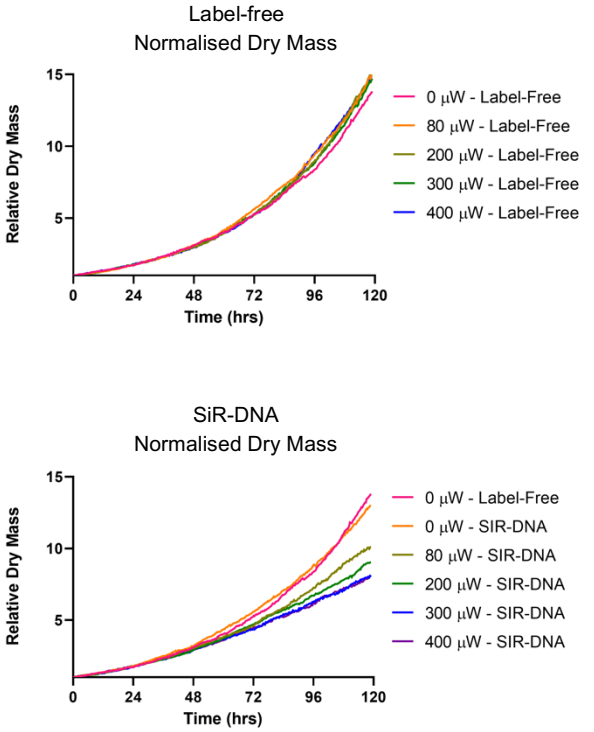


Figure 3: Relative dry mass over time for unlabelled (top) and SiR-DNA-labelled (bottom) cells at varying irradiation doses. SiR-DNA labelling combined with irradiation inhibits the overall accumulation of dry mass per field of view.

Total Dry Mass

Cellular dry mass is a metric unique to QPI and represents the total mass of all cellular components, including proteins, lipids, carbohydrates and DNA, amongst others but excluding water [6,7]. The total dry mass per field of view is automatically calculated by Livecyte providing a direct read-out of the total cellular mass in each image. This metric combines the effects of both individual cell growth between divisions and proliferation of the cell population.

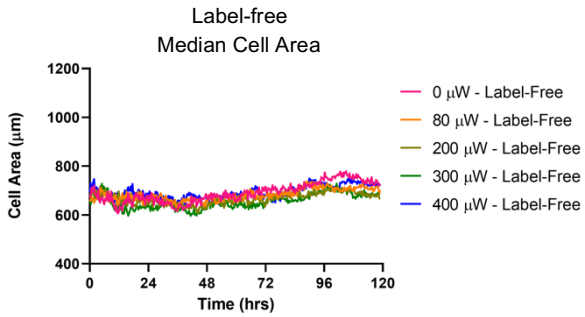
Relative dry mass per field of view was shown to be unperturbed by irradiation dose alone, with comparable increases in mass observed, over time, at all power exposures (Figure 3). However, the combination of SiR-DNA and irradiation was shown to elicit an irradiation-dose-dependent inhibition of dry mass accumulation, relative to controls (Figure 3). This indicates that the interaction between the SiR-DNA label in the LED irradiation causes the observed changes in cell growth and proliferation rates.

© Phase Focus Limited. AN012 | May 2019

phasefocus

Single Cell Area & Dry Mass

The high-contrast label-free images generated by Livecyte allows for robust individual cell segmentation during long-term time-lapse experiments. The combination of cell segmentation and QPI information provides a suite of morphological metrics that include cell area, perimeter, thickness, dry mass and sphericity.



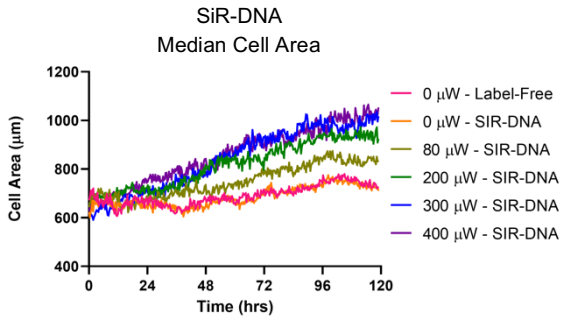
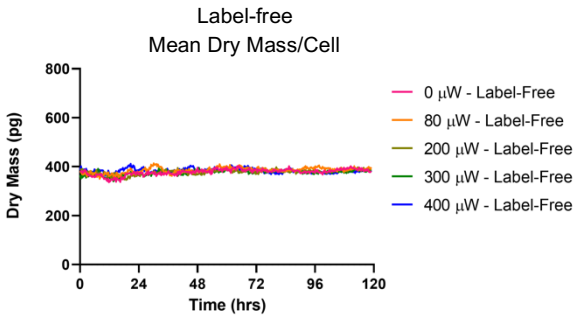


Figure 4. Median cell area of unlabelled (top) and SiR-DNA-labelled (bottom) cells at varying irradiation doses. SiR-DNA labelled cells exhibited an increase in median cell area over time.



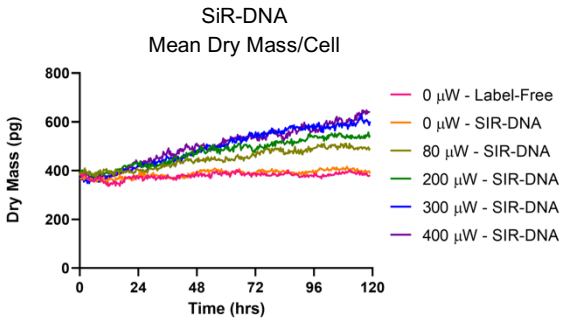


Figure 5: Plot of mean dry mass per cell for unlabelled (top) and SiR-DNA labelled (bottom) cells. The average dry mass of individual SiR-DNA-labelled cells increased over time, relative to unlabelled cells.

Median cell area increased over time in cells labelled with SiR-DNA and irradiated, in a dose-dependent manner (Figure 4). Increases in cell area also correlated with an increase in average dry mass per cell in labelled and irradiated groups (Figure 5), suggesting an accumulation in cellular components in response to interaction between the fluorescent label and LED excitation. No significant changes in cell area or mean dry cell mass were observed in control cells.

© Phase Focus Limited. AN012 | May 2019

phasefocus

Summary

The phototoxicity and potential off-target effects of blue and green excitation probes have been reported previously [3, 4]. A recent study demonstrated that far-red DNA probes can induce DNA damage and impair cell cycle progression [7], yet, the long-term effects of these probes are relatively unexplored.

In this study we show that MDA-MB-231 cells labelled with far-red dye SiR-DNA and exposed to common fluorescence illumination levels exhibit different phenotypic behaviour to unlabelled cells, even within 24 hours. The labelled cells exhibit reduced proliferation and growth as well as increased individual cell area and dry mass when compared to unlabelled controls.

The results highlight how off-target effects caused by fluorescent labels can be monitored with Livecyte to identify their impact on live cell assays. We also conclude that great caution must be taken when using fluorescent labels in any live cell experiments, even if those labels are perceived to be “live cell friendly”.

References

1. Iford R, Simpson HM, Duberman J, Hill GC, Ogawa M, Regino C, Kobayashi H, Choyke PL (2009). Toxicity of organic fluorophores used in molecular imaging: literature review. *Mol Imaging* 8(6):341-54.
2. Coutu DL, Schroeder T (2013). Probing cellular processes by long-term live imaging-historic problems and current solutions. *J Cell Sci.* 126(Pt 17):3805-15.
3. Magidson V, Khodjakov A (2013). Circumventing photodamage in live-cell microscopy. *Methods Cell Biol.* 114:545-60.
4. 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
5. Kasprowicz R, Suman R, O'Toole P 2017. Characterising live cell behaviour: Traditional label-free and quantitative phase imaging approaches. *Int J Biochem Cell Biol* 84:89-95.
6. Barer R 1952. Interference microscopy and mass determination. *Nature* 169(4296):366-7.
7. Sen, Onur, Adrian T. Saurin, and Jonathan MG Higgins (2018). The live cell DNA stain SiR-Hoechst induces DNA damage responses and impairs cell cycle progression. *Sci Rep* 8(1):7898.

© Phase Focus Limited. AN012 | May 2019

phasefocus

phasefocus

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.vimeo.com/phasefocus

T: +44 (0)114 286 6377
E: enquiries@phasefocus.com

Phase Focus Limited
Electric Works
Sheffield Digital Campus
Sheffield
S1 2BJ
UK

www.phasefocus.com