NEW NONTOXIC NUCLEAR PROBE TO STUDY LONG-TERM CHANGES OF CELL POPULATIONS

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Overview

- Nuclear staining allows for precise measurements of cell count, heath, and proliferation, which are pivotal in understanding disease biology and therapy development.
- Yet, current staining methods show a number of limitations:
  - Lentiviral transfection is time-consuming with limited cell line applications;
  - Toxic nuclear dyes used in endpoint assays cannot be directly combined with cell count/health assessments;
  - Nontoxic nuclear dyes for live cell show toxic effects even at their recommended low concentrations.
- We present a novel non-toxic biocompatible nuclear probe, NucleoLIVE, that allows for extended monitoring of cell proliferation and viability.

Introduction

In this study, we demonstrate the properties of NucleoLIVE in terms of:
- Non-toxicity;
- Segmentation capabilities;
- Compatibility with other assays.

As a mix-and-read reagent, the nuclear probe simplifies automation workflows. Below, we present the spectral and staining properties of NucleoLIVE on MCF-7 cells.

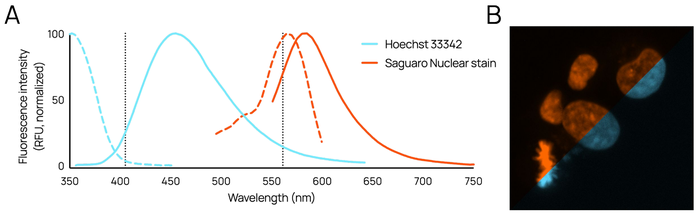

Figure 1. Nuclear dye spectral properties and imaging examples. A. Fluorescence spectra of MCF-7 cells stained with Saguaro's NucleoLIVE, relative to the Hoechst 33342 nuclear dye. Vertical lines represent 405nm and 561nm laser excitations. **B.** MCF-7 nuclei stained with either NucleoLIVE dye (1x, orange) or Hoechst 33342 (blue).

Primary Validation of Non-Toxicity

MCF-7 cells were transfected with the Nuclight Green Lentivirus (Sartorius) to express a non-perturbing nuclear restricted green fluorescent label. This label was used as ground truth in the toxicity assay of NucleoLIVE. The toxicity assay, using Incucyte S3, consisted of an imaging assay to count nuclei. All three dilutions of NucleoLIVE show no effect on cell proliferation over 6 days.

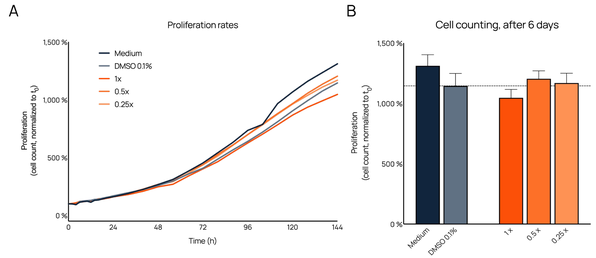

Figure 2. Proliferation assay: normalized cell count over 6 days. A & B. Cell counts were normalized to baseline timpoint cell count (t0). The "Medium" assay corresponds to the ground truth with the stably expressing fluorescent protein. All three recommended working concentration of NucleoLIVE show no toxicity relative to the "Medium" and DMSO 0.1% assays at every timepoint collected.

Segmentation

With clear and specific nuclear staining and high signal-to-noise ratio, NucleoLIVE is ideal for cell segmentation in bio-image analysis. In addition, NucleoLIVE's biocompatibility allows for real-time cell segmentation in kinetic assays.

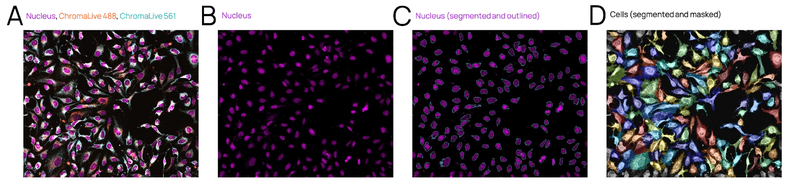

Figure 3. Investigating the segmentation capacities of the Saguaro nuclear dye, NucleoLIVE.

Compatibility with Other Assays

A small study conducted with Saguaro's new non-toxic LIVE/Dead Stain served as a proof-of-concept for showing the compatibility of NucleoLIVE with other fluorohpores. In this specific use-case, the LIVE-Dead stain can quantify cell death and viability in real-time, and by using a non-toxic nuclear dye, cell counts can be measured, providing an additional dimension that can help better distinguish compound mechanisms.

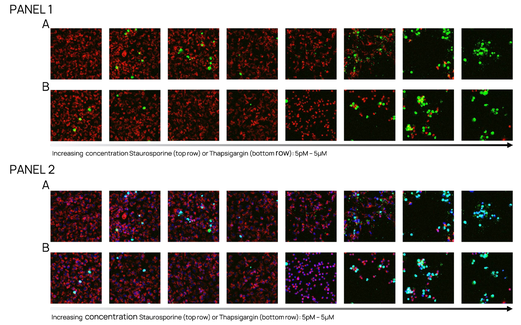

Figure 4. MCF-7 cells stained with Saguaro LIVE/Dead stain and with or without Saguaro's NucleoLIVE. The two panels show LIVE/Dead stain without NucleoLIVE (panel 1) and with nuclear dye (panel 2). For both panels, live cells in red channel and dead cells in green channel. For panel 2 only dead cells also appear aqua, and nuclei in blue. For both panels: **A.** Cells are treated with increasing doses (left to right) of Staurosporine and imaged at 48h. **B.** Cells are treated with increasing doses (left to right) of Thapsigargin and imaged at 48h.

Conclusion

The new nuclear probe represents a considerable advancement in drug discovery and cell biology tools, helping researchers with a new level of detail of drug effects and disease biology:

- Non-toxicity allowing real-time cell counting and long-term proliferation assays with constant labelling of cells as they divide;
- Rapid and easy mix-and-read protocol;
- Allow live-cell segmentation in kinetic assays;
- Can be multiplexed with other live cell fluorophores.