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Trypan blue staining does not interfere with the emission spectra of fluorescent dyes in confocal microscopy

Mia Stanić[1*]

[1]*Department of Laboratory Medicine and Pathobiology, Faculty of Medicine, University of Toronto, MaRS Centre, West Tower, 661 University Avenue, M5G 1M1, Toronto, Ontario, Canada.*

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***Correspondence**

E-mail: mia.stanic@utoronto.ca

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The dye exclusion assay is a fast and cheap way to determine the number of viable cells in a population of cells. The assay is based on the fact that viable cells possess intact plasma membranes that disable the penetration of dyes such as trypan blue, propidium iodide, or other DNA intercalating dyes. In contrast to dead cells which have perforated plasma membranes and can absorb mentioned dyes (Strober, 2015).

For a multitude of experimental setups it is crucial to understand the viability of cell populations, especially when working with DNA damage inducing agents (Aslantürk, 2017). Moreover, occasionally there is a requirement to prepare multiple samples from the same population of cells, including the fraction for a viability test, especially when the amount of starting material is limited. In that sense, multiplexing or combining multiple tests and assays in one sample can be advantageous (Van Gool et al., 2020). The trypan blue test is probably the most commonly used assay for a quick estimation of cell viability (Mishell and Shiigi, 1980). However, multiple lines of evidence show that the simultaneous application of trypan blue staining together with another fluorescent dye can impair sample acquisition and analysis. Accordingly, it has been shown that trypan blue can quench green fluorophores (Shilova et al., 2017), or that it can emit fluorescence in the far-red spectrum as seen in flow cytometry (Mosiman et al., 1997). Reports from yeast studies show that trypan blue can interact with cellular proteins and emit strong red fluorescence (Liesche et al., 2015).

This note demonstrates that a combination of staining procedures composed of trypan blue staining and classical immunofluorescence in the human U2OS cell line does not impair the quality of confocal microscopy. The trypan blue staining does not interfere with the emission spectra of ultraviolet (Hoechst), green (Alexa 488) and red dyes (Alexa 568), respectively. Moreover, the simultaneous staining procedures together give information about cell viability, as well as information about other analyzed targets via microscopy in the same sample.

For sample preparation U2OS cells were seeded on glass coverslips to reach 70% confluency. Afterwards the cells were washed in 1X PBS and covered for 1 minute in a 0.4% trypan blue/1X PBS

solution (v/v). After incubation with the trypan blue containing solution, the cells were rinsed 1X PBS and fixed, permeabilized and immunofluorescent (IF) stained as previously described by Marini et al., 2015. IF stained cellular targets included in the analysis were Topoisomerase 2α and DNA double strand break marker γ-H2AX (Mah, El-Osta, & Karagiannis, 2010). Topoisomerase 2α was included in the analysis considering its diffused staining pattern. On the other hand, because of its mainly dotted staining pattern in healthy cells γ-H2AX was selected, in order to compare a potential interference of the trypan blue staining with different staining patterns of nuclear proteins. Finally, the cells were also counterstained with Hoechst nuclear stain in order to assess a potential interference with multiple fluorescent dyes of different emission spectra (Figure 1). The obtained results after staining, imaging and data analysis show that the targets analyzed in the UV, green or red laser spectrum do not show any interference or bleed-trough of the trypan blue staining (Figure 1). The results indicate that the trypan blue staining can be combined with the IF staining of nuclear proteins without interfering with the emission spectra of certain secondary antibodies. Moreover, the results show that trypan blue does not interact with nuclear proteins and does not affect their staining patterns in U2OS cells. Ultimately, a combination of trypan blue staining and classical IF staining procedures of cells can provide important insights into population viability, as well as target protein assessment in the same microscopy sample.

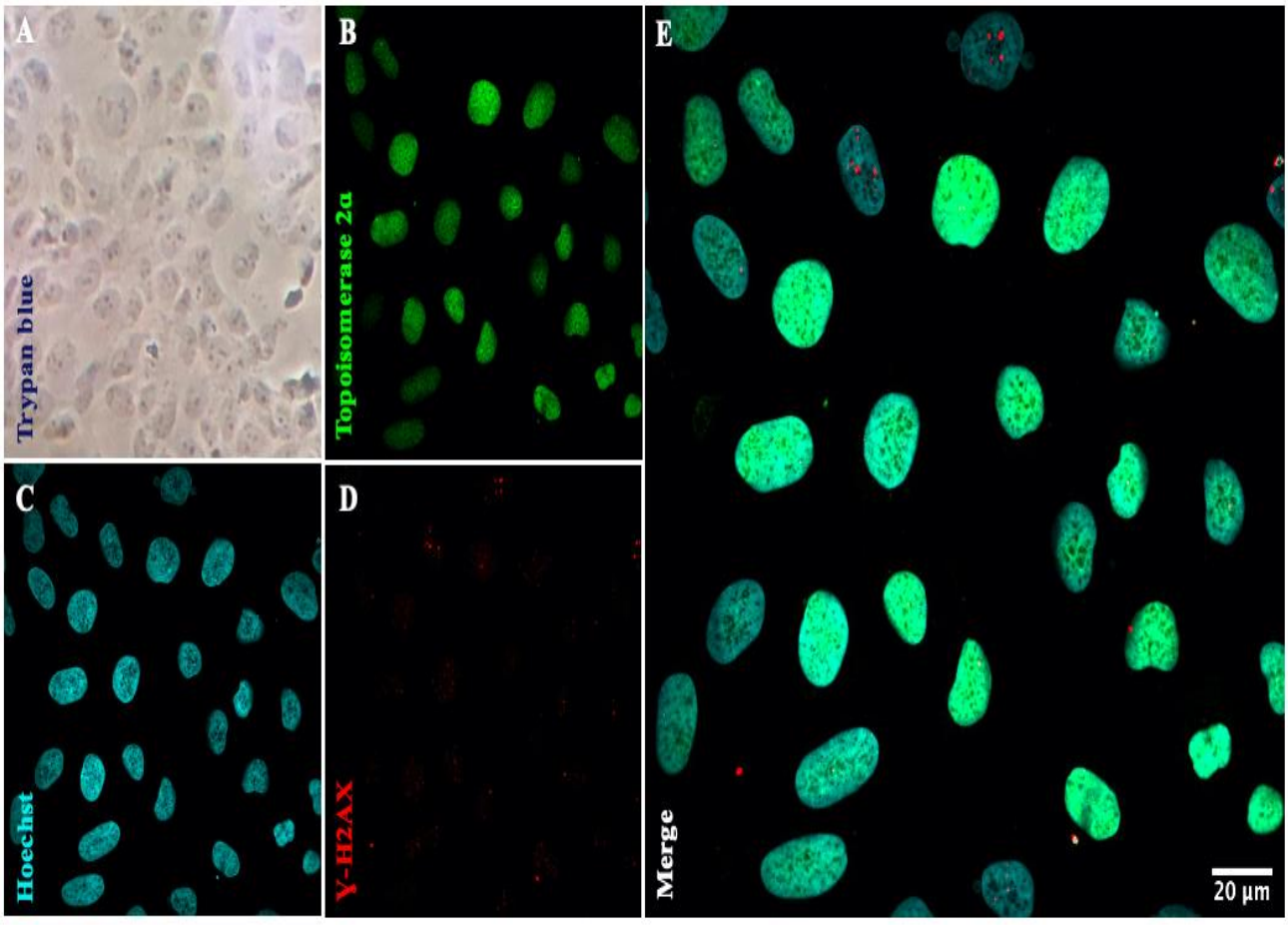

Figure 1. Microscopy images of simultaneous trypan blue and IF staining of U2OS cells. Panel A shows trypan blue staining of U2OS cells. Image was taken with a VE-403 inverted light microscope. Panels B-E show single plane confocal images of U2OS cells taken from the same microscopy slide as image in panel A. Confocal images were taken with a Nikon Eclypse ti2 microscope.

Conflict of Interest

The author declare no conflict of interest.

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