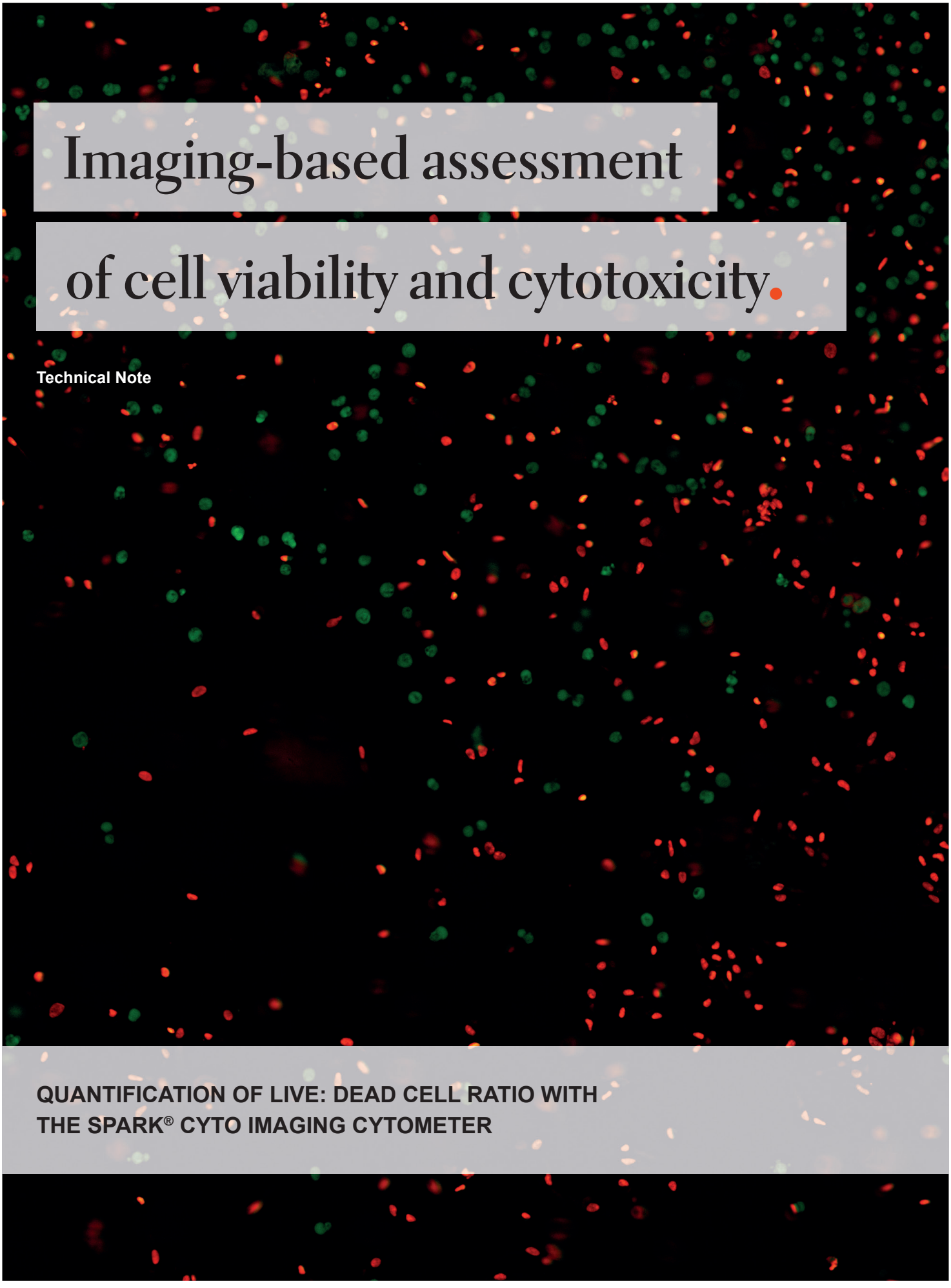
Imaging-based assessment of cell viability and cytotoxicity.

Technical Note



QUANTIFICATION OF LIVE: DEAD CELL RATIO WITH THE SPARK® CYTO IMAGING CYTOMETER

INTRODUCTION

Viability assays are commonly used in cell biology labs as a screening tool to assess cell line response to compounds of interest or different growth conditions. There are numerous methods for determining cell viability, some providing 'whole-well' information – eg. bioluminescent detection of ATP, or calorimetric assays using MTT, WST-1 or resazurin – while others use fluorescence-based imaging to discriminate between live and dead cells on an individual level. These fluorescent viability markers are usually combined with appropriate cell death markers based on membrane permeability and accessibility of the nuclei.

Tecan's new Spark Cyto combines the multimode detection capabilities of a microplate reader with a sophisticated camera module that enables bright field and fluorescence-based imaging of cell samples directly in the microplate well. This enables visual and functional examination of cell samples within a single experiment. The system's versatile and user-friendly SparkControl™ software includes a number of predefined protocols to simplify the set up of common cell-based assays. These include a Cell Viability application that uses calcein-AM in combination with propidium iodide (PI). Calcein-AM is a non-fluorescent, membrane permeable compound that is cleaved by the esterase activity in the cytoplasm of viable cells to yield the green fluorescent, membrane-impermeable compound calcein. In contrast, PI is membrane impermeable in viable cells, allowing for the detection of dead cells based on their red fluorescence.

This technical note describes a method for accurate and fast quantification of human cervical carcinoma (HeLa) cell viability following treatment with dimethylsulfoxide (DMSO) or saponin.

MATERIALS AND METHODS

HeLa cells were grown to confluence in RPMI medium (Gibco, #12633012) supplemented with 10 % fetal bovine serum (FBS) at 37 °C and 5 % CO_2 in a humidified atmosphere. Cells were then harvested using trypsin/EDTA, counted and seeded into a 24-well microplate at a cell number of $5x10^4$ cells per well in 500 µl culture medium. The cells were left to adhere at standard incubation conditions overnight.

In an initial experiment, DMSO was added to a final concentration of 20 or 40 % (v/v), and the cells were incubated for 45 min at 37 °C to induce cell death. In a second experiment, different concentrations of saponin (0.05 % and 0.2 % v/v in growth medium) were applied to the cells for 10 min at 37 °C. In both cases, untreated control wells were included.

For fluorescent staining, working solutions of calcein-AM and PI were prepared by diluting the stock solutions in PBS to final concentrations of 100 ng/ml and 1 pg/ml, respectively. The dyes were added directly to the cell samples (300 µl/well) and incubated for 30 min, in the dark, at room temperature. Wells stained with just PI were also included for crosstalk correction.

Image acquisition was performed using the Cell Viability application with the settings summarized in Table 1. Parameters like exposure time, LED intensity and focus offset were optimized in Live Viewer™ (accessible via Method Editor) to ensure sufficient fluorescence could be detected in each color channel, without overexposure. Crosstalk correction was performed by determining the green fluorescence signal of a reference well stained with PI only, and minimizing the amount of light in that channel.

Detection rates for green and red objects were optimized using the length, width and recognition sensitivity settings in Method Editor or Image Analyzer.

Parameter	Setting
Plate	TEC24ft_cell
Measurement mode	Fluorescence imaging
Application	Viability
Objective	10x
Pattern	Central
Green channel	
LED intensity	100 %
Focus offset	0 µm
Exposure time	60 ms
Sensitivity	90 %
Object size	4-45 µm width, 4-30 µm length
Red channel	
LED intensity	100 %
Focus offset	0 µm
Exposure time	200 ms
Sensitivity	95 %
Object size	4-28 µm width, 4-28 µm length

Table 1: Measurement settings for cell viability assessment using the green and red fluorescent channels.

RESULTS

Addition of 40 % DMSO resulted in a marked decrease of viability, along with an increase in the number of PI-positive cells from background level to around 50 %. However, a concentration of 20 % DMSO had minimal effect on this famously robust cell model (Figure 1). Moreover, the addition of 40 % DMSO caused the dying cells to detach from the well surface, potentially overestimating viability levels as is evident in figure 2 where overall less cells can be seen in the treated wells versus control wells.

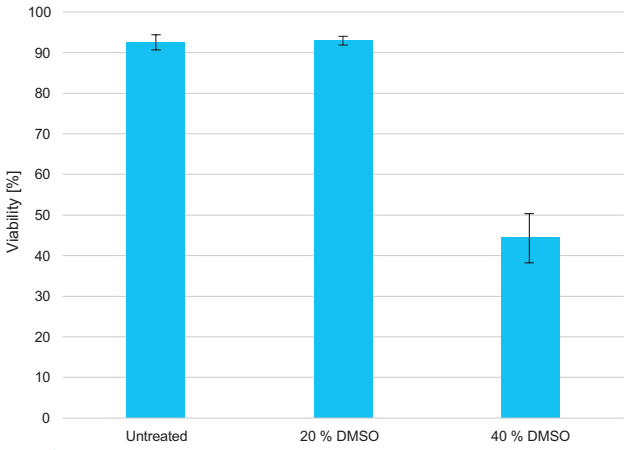


Figure 1: Viability assessment of HeLa cells treated with DMSO.

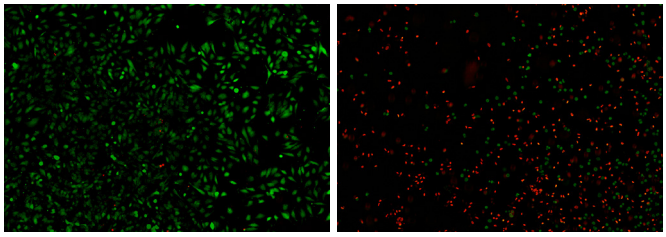


Figure 2: HeLa cells without DMSO treatment (left), and following treatment with 40 % DMSO (right). Following DMSO-induced cell death, calcein staining (green) is significantly reduced, and PI staining (red) is increased, showing cells have lost their membrane integrity.

In contrast, relatively low concentrations of saponin caused immediate loss of membrane integrity without de-taching cells from the well surface. Treatment resulted in a dramatic reduction in viability, to approximately 10 % with 0.2 % saponin (Figure 3) and the majority of cells staining positive for PI (Figure 4).

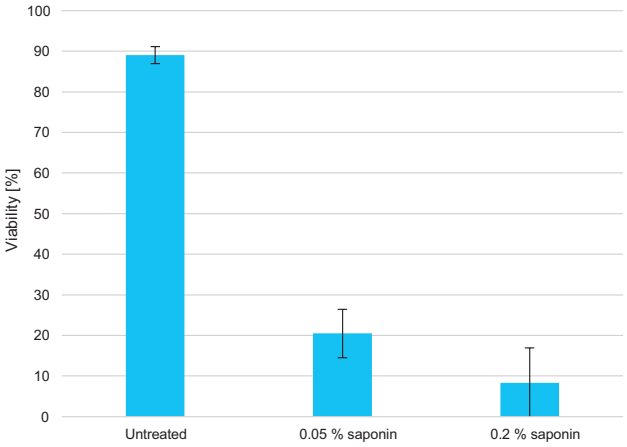


Figure 3: Viability assessment of HeLa cells treated with saponin.

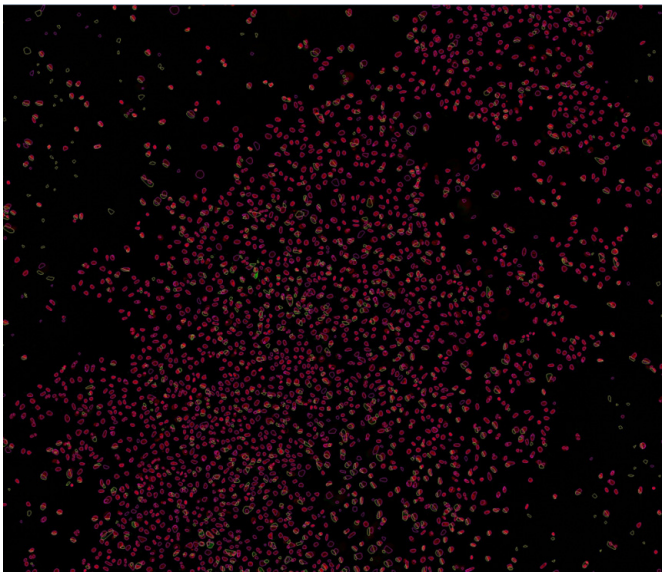


Figure 4: Object recognition and discrimination using Image Analyzer software (cells treated with 0.05 % saponin).

Cell viability assessment can also be performed over time, ie. as a kinetic measurement. For this application, the use of environmental control features – such as temperature and humidity control, as well as appropriate CO_2 partial pressure for the chosen cell line – is recommended. However, depending on the cell line, it should also be noted that calcein is actively transported out of the cells several hours after staining.

CONCLUSIONS

Spark Cyto's predefined Cell Viability application provides a reliable and easy-to-use method for detecting and quantifying the cytotoxic effects of compounds, in this case the effects of DMSO or saponin on HeLa cells. The system's Image Analyzer software allows straightforward optimization of the recognition sensitivity for green and red objects. Furthermore, overexposed objects or artifacts can be excluded by gating by size or intensity.

ABBREVIATIONS

AM	acetoxymethyl
DMSO	dimethylsulfoxide
LED	light-emitting diode
PBS	phosphate-buffered saline
PI	propidium iodide

About the authors

Dr. Nicole Eggenhofer is an application specialist at Tecan Austria. She studied genetics at the University of Salzburg and focused on cell biology and microbiology during her Ph.D. Nicole gained further experience in the field of molecular biology before joining Tecan in 2017. She has been involved in the development of Spark Cyto.

Dr. Katrin Flatscher is an application specialist at Tecan Austria. She studied molecular biology at the University of Salzburg and focused on cell biology and immunology research during her PhD. She joined Tecan in 2007 and has been involved in the development of the Infinite as well as the Spark multimode reader series.

Dr. Lynda O'Leary is an application specialist at Tecan Austria. She received her PhD in Biochemistry at the National University of Ireland, Galway, working in the field of apoptosis and cell signaling. Following this, she joined the Institute of Cancer Research in London as a postdoctoral fellow focusing on metastatic dormancy in breast cancer. Lynda has extensive experience in a wide range of molecular cell biology techniques, cell culture and imaging. In 2018, she joined the sales and marketing department with a primary focus on the Spark Cyto multimode microplate reader.

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Tecan has not independently validated this method with all possible sample types or analytical uses and is providing this example method as a convenience to users. The user must independently evaluate and validate: (a) the suitability of the method for their use, (b) their ability to process samples of their choosing following the method; and (c) their ability to proficiently perform the method in their facility with their personnel.

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