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Holographic analysis of single cells

native and staining-free pharmacologic assessment of drug dose responses

Introduction

A sensitive and reproducible cell viability assay is essential for monitoring cell health in cell culture laboratories. Moreover, viability assays are a fundamental tool in the drug discovery process as well as in the assessment of cytotoxicity. Assaying cell viability typically involves biochemical methods, such as dye exclusion stains to probe membrane intactness, DNA intercalating agents or metabolic assays that emit fluorescence based on enzymatic activity [1, 2]. However, this involves additional incubation and handling steps of mostly cytotoxic reagents leading to additional error sources, and prolonged experimental time.

In contrast, the fluidlab R-300 detects viability based on morphological and foremost compositional changes occurring during cell death. The change in composition e.g. reflects protein turn-over and can be assessed due to the quantitative nature of the phase signal in the fluidlab R-300's innovative holographic microscope. This technological novelty makes additional handling and staining steps unnecessary and the percentage of viable cells is directly accessible from a cell suspension.

In this proof of concept study, we compared the performance of the label-free viability tool of the fluidlab R-300 to two well-established colorimetric viability readouts. Colorimetric viability assays use a variety of markers as indicators of metabolically active (living) cells. The cell-permeable reagents are reduced by metabolically active cells to strongly coloured reporter molecules, whose fluorescent signal is proportional to the number of living cells in the sample. These colorimetric assays are commonly used as a screening method to determine the cytotoxic potential of drugs. We show that the staining-free viability tool of the fluidlab R-300 can be reliably used for monitoring cell health and for assessing dose response curves in pharmacology.

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Methods

Cell culture and preparation

Cells were plated at a density of 1500 cells per well in triplicate in 96-well plates. Cells were allowed to attach and grow at 37°C, 5 % CO_2 for 24 h. Subsequently, the small molecule drugs staurosporine or GI254023X were added to cells in triplicate wells, at concentrations ranging from 0,1 nM to 1000 nM (for staurosporine) or 1 µM to 1000 µM (for GI254023X). Cells were treated for 4 days at 37°C, 5% CO2.

Cell lines used	
U87MG	cancer cells
MCR5	fibroblasts
HL-1	cardiomyoctes

Cell viability assays

To assess cell viability, two colorimetric assays (MTT and resazurin based assays) were compared to the staining-free viability tool of the fluidlab R-300. For the colorimetric cytotoxicity assays, medium was replaced with 100 µl/well MTT solution (final concentration 1 mg/ml in RPMI medium) or 20 µl µl of resazurin solution (final concentration 0.015 mg/ml) was directly added to 200 µl culture medium per well. After incubation with the dye for 4 hours, absorbance was read out at 570 nm with a BioTek Synergy HTX Multi-Mode microplate reader.

For the staining-free viability assay, cells were detached from the wells by adding 30 µl of trypsin for 4 min. The trypsin reaction was stopped by adding 30 µl of culture medium. For each replicate, 20 µl of cell suspension was loaded into the acella100 sample carrier and viability was assessed using the fluidlab R-300.

For all three assays, cell viability was normalized to the viability of the untreated DMSO control sample.

Material	Reagents
fluidlab R-300	cell culture medium
acella100 sample carrier	thiazolyl blue tetrazolium bromide (MTT)
microplate reader	resazurin cell viability kit
96-well plates	staurosporine, GI254023X
	trypsin

Results

Cell death was induced by treating three different cell lines with small molecule drugs that cause cell death through apoptosis. Triggering of apoptosis causes specific molecular changes together with morphological changes, such as cell shrinkage, membrane fragmentation and nuclear condensation. These changes in protein content and cell morphology can be visualized by the state-of-the-art digital holographic microscopy method used in the fluidlab R-300[3]. Every cell in the sample is analyzed by convolutional neural networks to determine whether it is dead or alive. Live cells are characterized by a dark contour and a structured cytoplasm, while dead cells lose their well-defined boundary and appear black. Figure 1 shows exemplary cell images obtained with the fluidlab-R300.

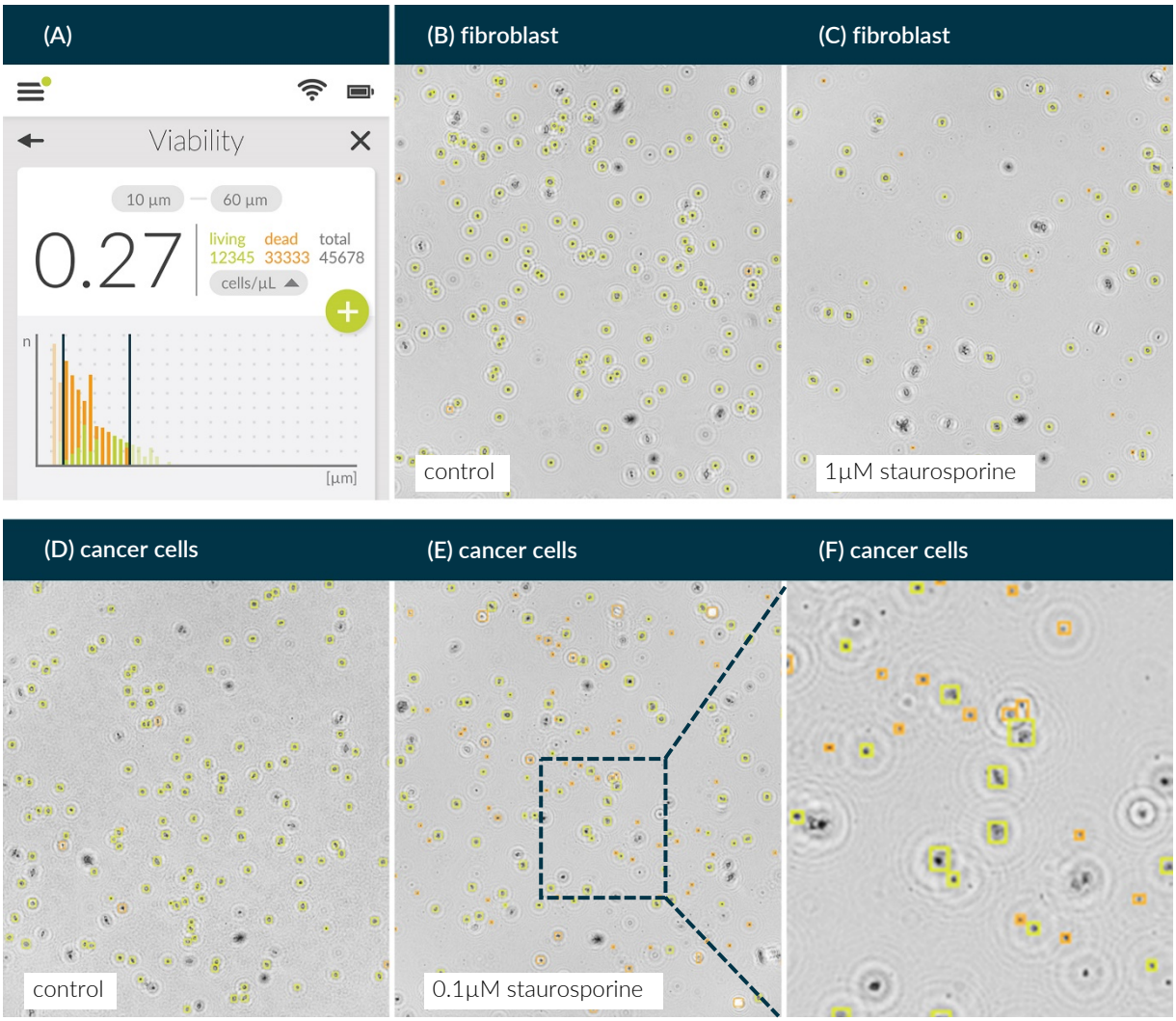
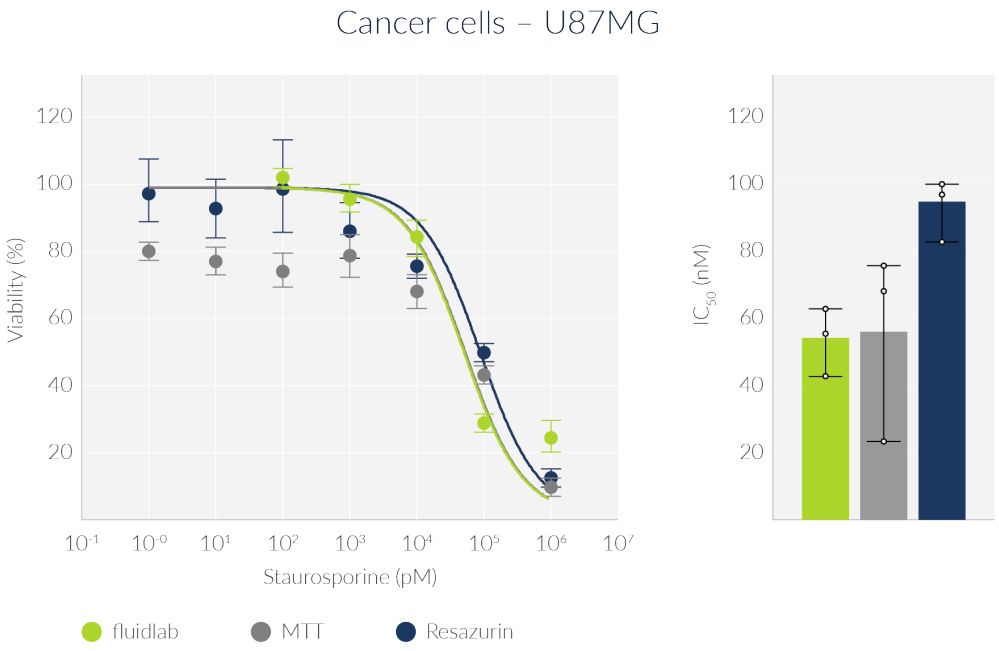
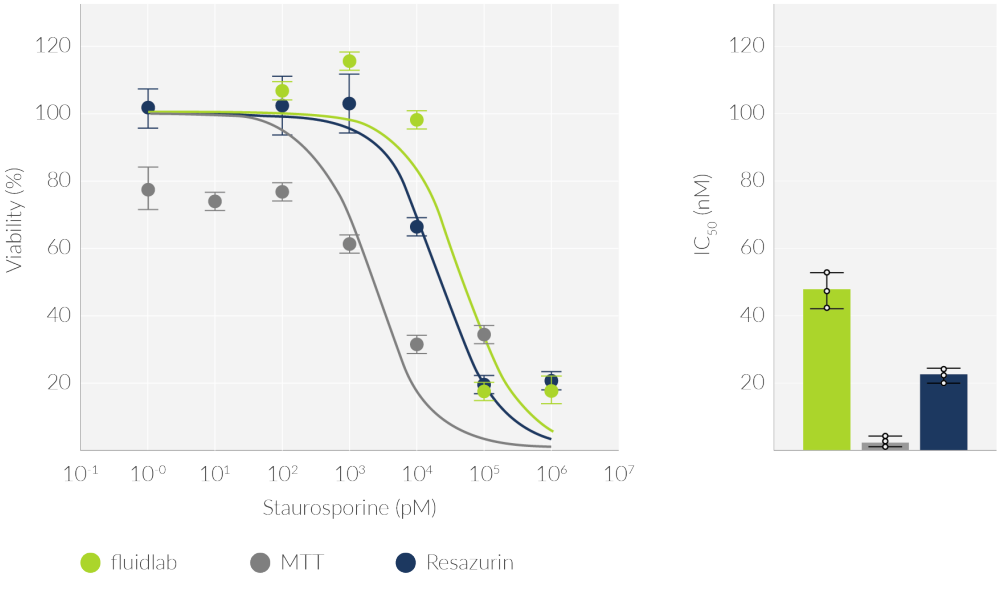


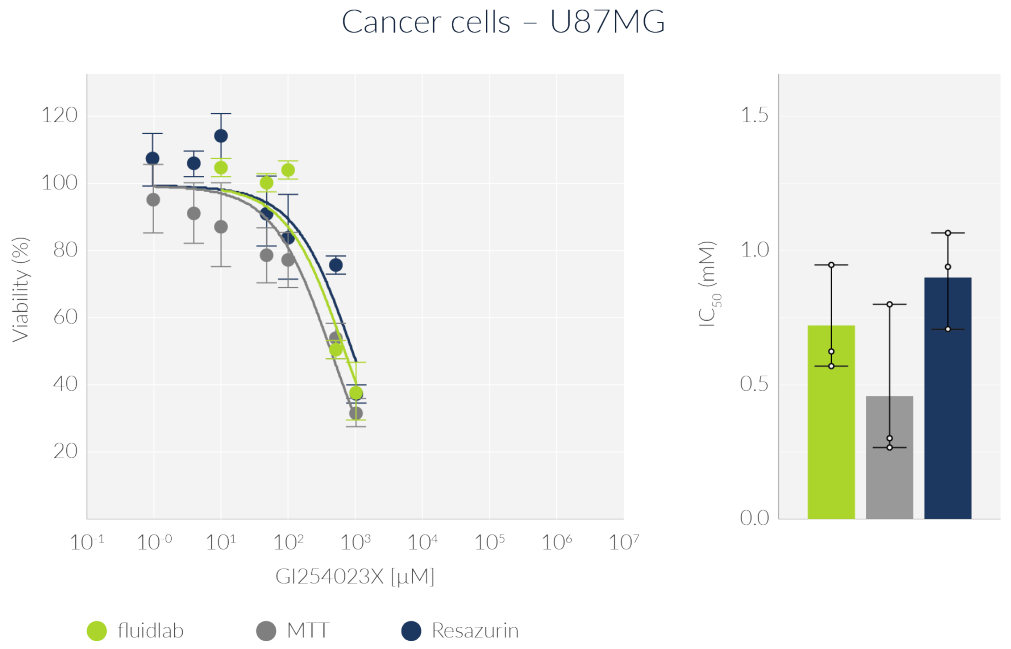
Figure 1: *Staining-free viability measurements with the fluidlab R-300. (A) Result screen of the fluidlab R-300 showing the percentage of viable cells and the total cell count for dead and alive cells. Representative images of (B) control fibroblasts (MCR5) cells, (C) fibroblast cells treated with 1 µM staurosporine, (D) control cancer cells (U87MG) and (E) cancer cells treated with 0,1µM staurosporine. Live and dead cells are marked with green and orange boxes, respectively. (F) Zoom in on the region labeled with the blue rectangle in (E) to show the distinct morphology of dead and alive cells.*

[3] Method: Staining-free Viability Explained. https://anvajo.com/storage/media/documents/1076/fluidlab_viability_explained.pdf

Cell viability was measured using the staining-free viability tool of the fluidlab R-300 and two colorimetric assays (MTT and resazurin viability assay). The percentage of viable cells in the sample was monitored at different drug concentrations. Treatment of all three cell lines with staurosporine–a general protein kinase inhibitor and potential anti-cancer therapeutic–showed a concentration-dependent decrease in the percentage of living cells (Figure 2). Additionally, cancer cells (U87MG) were treated with GI254023X, a metalloproteinase inhibitor inducing apoptosis. All three viability assays show comparable dose-response curves for the three different cell lines tested. By fitting the dose-response curves with a sigmoidal function, the half-maximal inhibitory concentration (IC50) for each assay was determined. According to the IC50 values, cardiomyocytes (HL-1) were more sensitive to staurosporine than cancer cells (U87MG) and fibroblasts (MCR5). The staining-free viability tool of the fluidlab R-300 proved to perform similarly as the colorimetric assays.







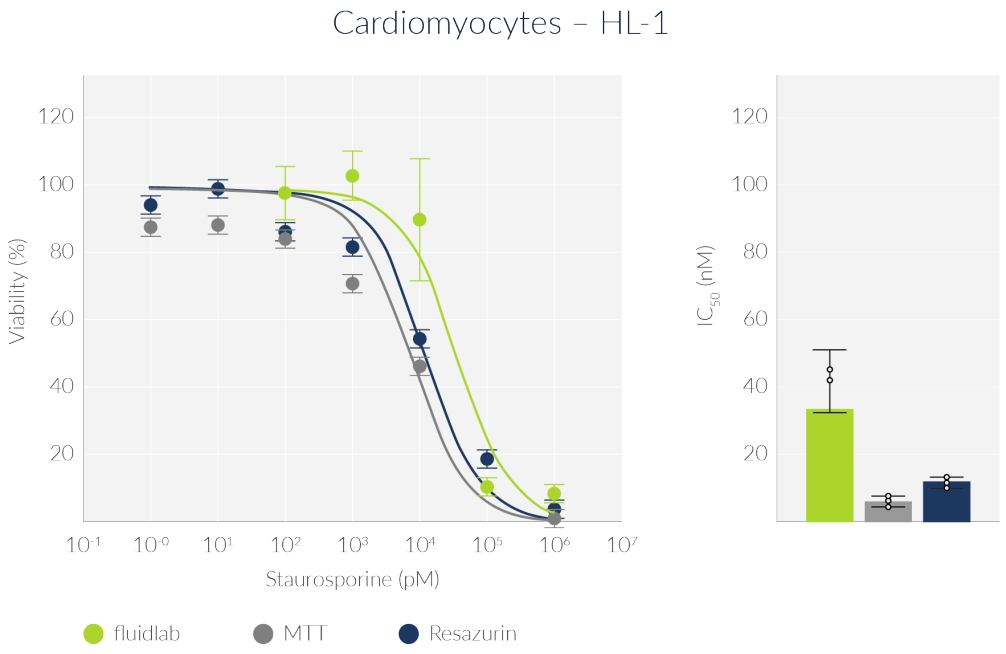


Figure 2: *Comparison of different cell viability assays for drug screening. Dose-response curves were measured for two different drugs (staurosporine and GI254023X) for three different cell types. The percentage of viable cells and IC50 values were determined using the fluidlab R-300 (green), the MTT assay (grey) and the resazurin assay (blue). Averages and standard errors of the mean were derived from triplicates.*

Assay	staurosporine IC50 (nM)		
	Cancer cells – U87MG	Fibroblasts – MCR5	Cardiomyocytes – HL-1
fluidlab R-300	54 ± 10	48 ± 5	34 ± 17
MTT assay	56 ± 29	2,2 ± 0,4	7 ± 2
resazurin assay	94 ± 7	23 ± 2	12 ± 1

Conclusion

Here, we showed that the staining-free viability tool of the fluidlab R-300 can be successfully employed for monitoring cell viability in the cell culture environment. The label-free analysis of the fluidlab R-300 was developed to work across a large range of cell types. Here, we showed that the fluidlab R-300 reliably detects cell viability for three different cell types with distinct morphology. We compared its performance to two colorimetric assays based on quantification of metabolic activity that are commonly used techniques in the drug screening process. While all three assays yield comparable results, the fluidlab R-300 offers the distinct advantage of assessing cell viability without introducing fluorescent dyes into the culture. In contrast, the MTT and resazurin viability assays require pre-analytical staining and incubation steps, which are not just more time-consuming, but may also introduce artefacts due to staining. Long exposure of cells to dyes can induce cytotoxicity and thereby the assay itself may bias the quantification of the percentage of viable cells in a cell culture sample [1, 2]. Moreover, a variety of chemical compounds have been shown to interfere with MTT and resazurin reagents leading to significant conversion of the assay reagents even in the absence of any cellular activity [3].

In summary, the automated imaging and quantitative analysis approach of the fluidlab R-300 allows for the testing of cytotoxic compounds and is suited for the assessment of cell viability in numerous biological assays without the need for additional staining.

References

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