

>> Long-term, noninvasive viability monitoring of paclitaxel treated cells

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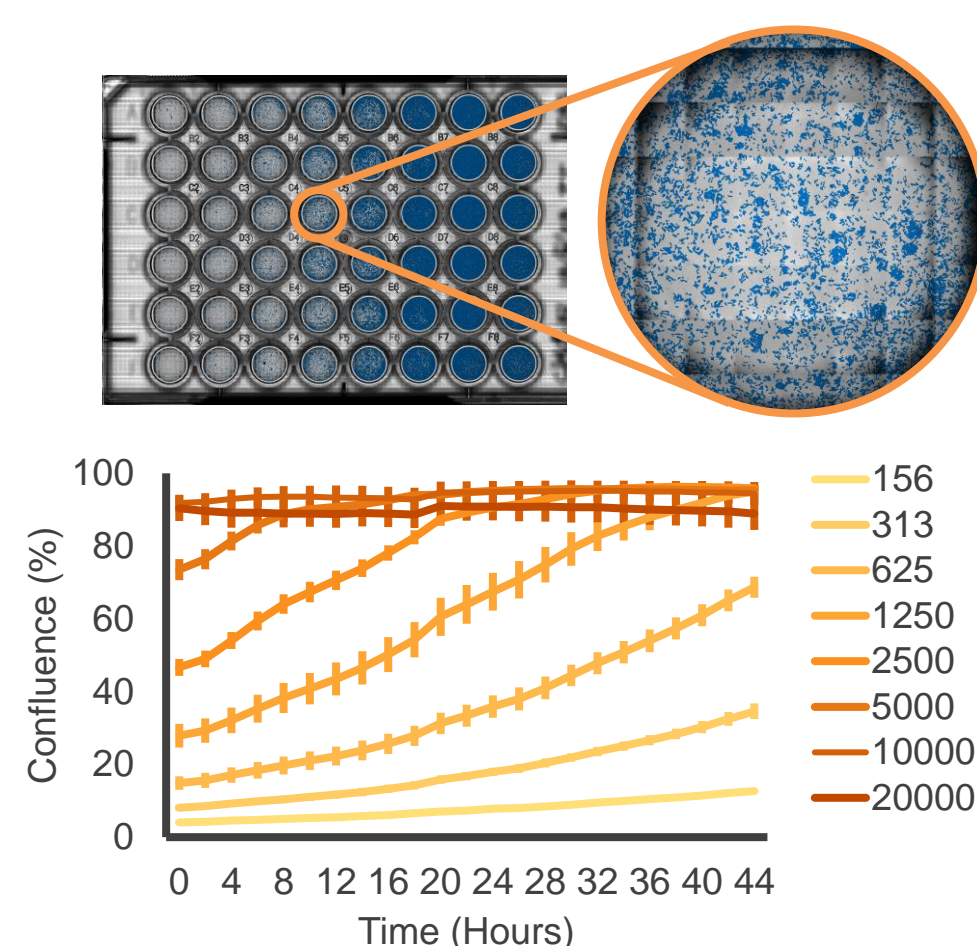
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Omni: Kinetic viability tracking

Automated, whole-vessel imaging and analysis

Cell viability and cytotoxicity assays are often used to understand the mechanism and potency of novel drugs or cell therapies. Assessing the degree and dynamics of the target cell response *in vitro* is vital, but endpoint assays only provide a snapshot and can miss key indicators of cellular response. Therefore live-cell imaging provides a noninvasive alternative as a measure of cytotoxicity.

Axion BioSystems' Omni platform offers live-cell imaging within an incubator for real-time tracking of cell proliferation, migration, colony formation, and organoid size. Here, we used the Omni to assess the long-term cytotoxic properties of a known compound on C6 rat glioma cells.



The Omni Product Family



- >> **Assay your cells in brightfield and fluorescence** – From label-free cell monitoring to fluorescence-based assays, the Omni adds dynamic visual results to any experiment.
- >> **Track every moment, straight from your incubator** – Operating within an incubator, the Omni automatically captures images as your cells grow in their optimal environment.
- >> **See every cell** – The Omni moves the camera, capturing detailed brightfield images of entire cell cultures without disturbance.
- >> **Monitor and analyze your cells remotely** – The software allows you to monitor your cells and perform data analysis from your desktop.
- >> **Get started quickly** – The Omni is easy-to-install, maintenance-free and does not require calibration, a short training is all it takes.

Features	Omni BR	Omni Pro 12	Omni FL
Whole-well brightfield	✓	✓	✓
Automated acquisition	✓	✓	✓
Incubator compatible	✓	✓	✓
Fluorescence (Red)		✓	✓
Fluorescence (Green)		✓	✓
Number of plates	1	12	1
Plate handling	Manual	Automated	Manual



AI-Driven Imaging Software for Powerful, yet Simple, Analysis

The Omni platform software modules simplify assay setup, offer real-time cellular visualization, and enable fast analysis. Find the ideal module for your research and transform complex data into clear results.



Cell Confluence



Scratch Assay



Fluorescent Object Count



Clonogenic Assay



Organoid Analysis

Real-time analysis of cell behavior

Brightfield confluency as a measure for cell viability

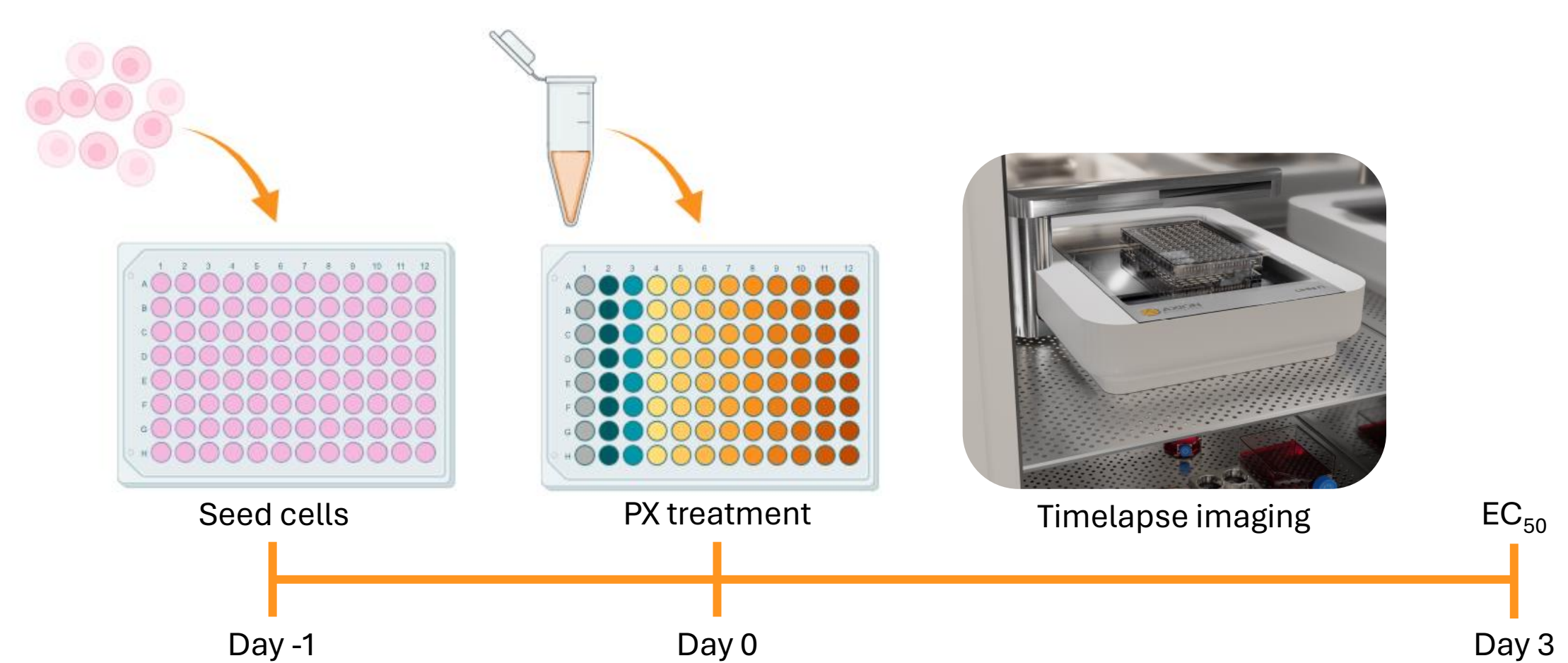
Introduction

Long-term cell viability monitoring is crucial in drug development, cancer research and toxicology. Traditional methods such as cell counting, live/dead viability staining, and metabolic activity assays are generally invasive end-point measurements and thus unsuitable for long-term viability monitoring. An alternative approach is to assess viability by measuring confluency through intermittent brightfield microscopy of cultured cells. This label-free method allows for long-term viability tracking and observation of morphological changes preceding cell death. Automated live-cell imaging enables non-invasive analysis of multiple samples simultaneously in a CO₂-incubator, ensuring optimal conditions.

In this study, we aimed to demonstrate the feasibility of using non-invasive confluency measurements as a measure of viability, specifically through confluency analysis of Paclitaxel treated glioma tumor cells.

Methods

C6 cells (rat glial tumor cells) were cultured in a 96-well plate for 24 h. Thereafter, Paclitaxel (PX) – a chemotherapeutic drug that interferes with normal microtubule function - was added at concentrations ranging from 1 to 1000 nM and a high-resolution scan of the well plate was made every hour with the Omni live-cell imager (37°C and 5% CO₂; n = 8 per group). The confluency of each well was determined at each timepoint and normalized for the confluency at 0 h to account for differences in confluency at the start of the experiment. Next, the half-maximal effective concentration (EC₅₀) at 72 h was calculated by fitting a Hill equation to the dose response curve at that timepoint.



Experimental workflow: Cells were cultured for 24 hours before addition of PX. High-resolution scans were made hourly for 72 hours using the Omni.

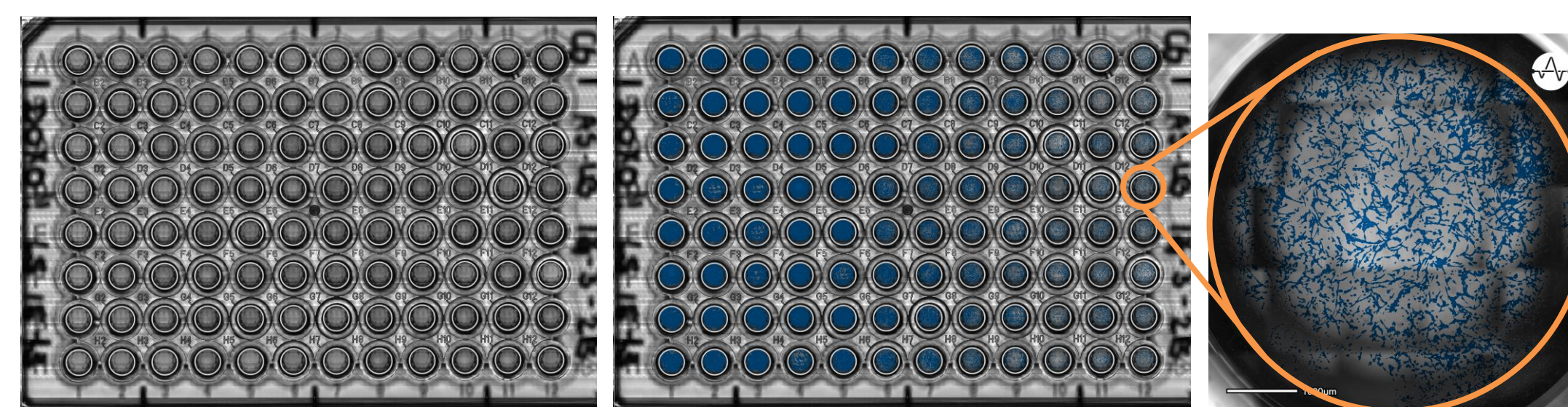


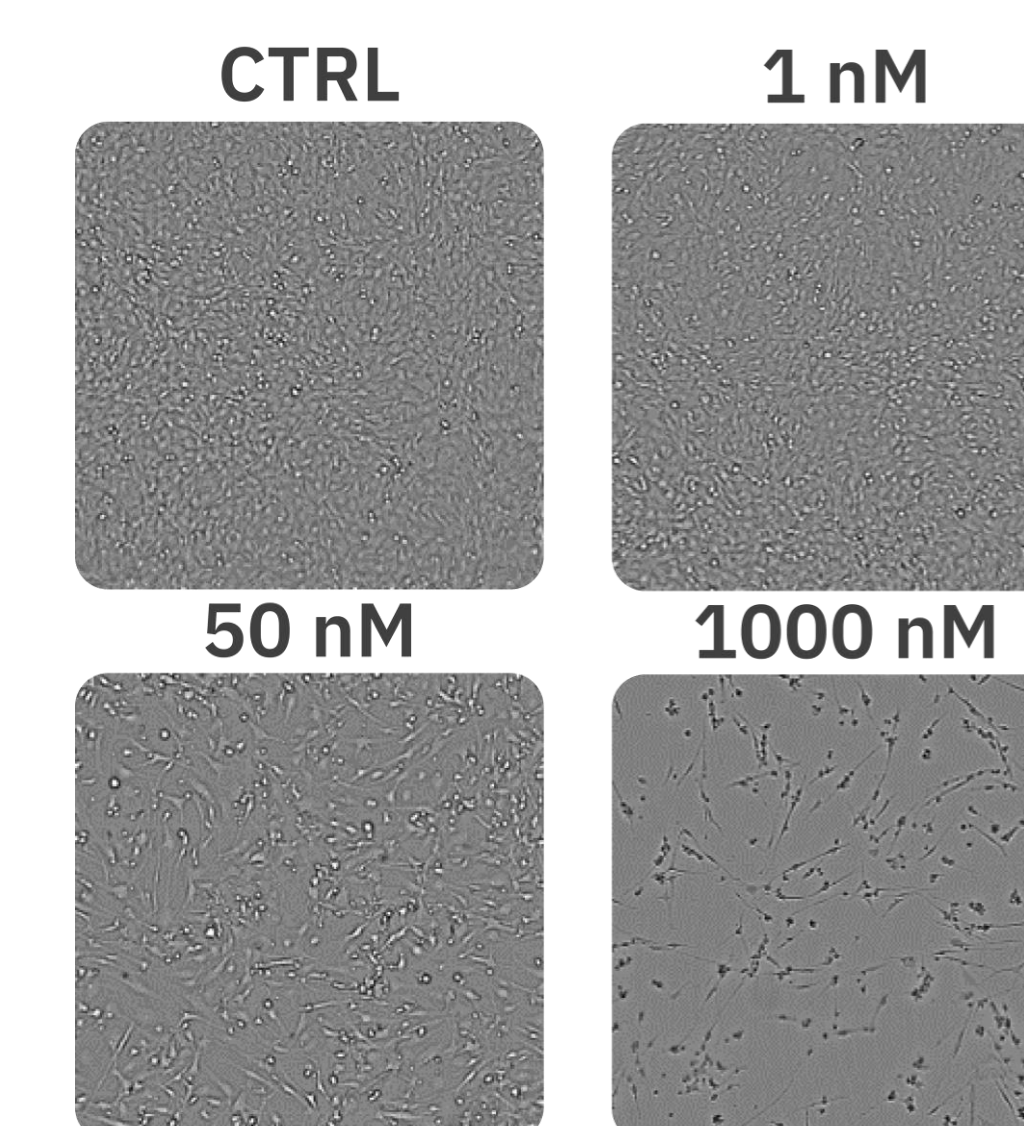
Image analysis workflow: high-resolution scans made with the Omni were analyzed using the confluency module available in the Axion Portal.

Dynamic insight into cell viability

Paclitaxel induces a dose-dependent decrease in C6 cell confluency

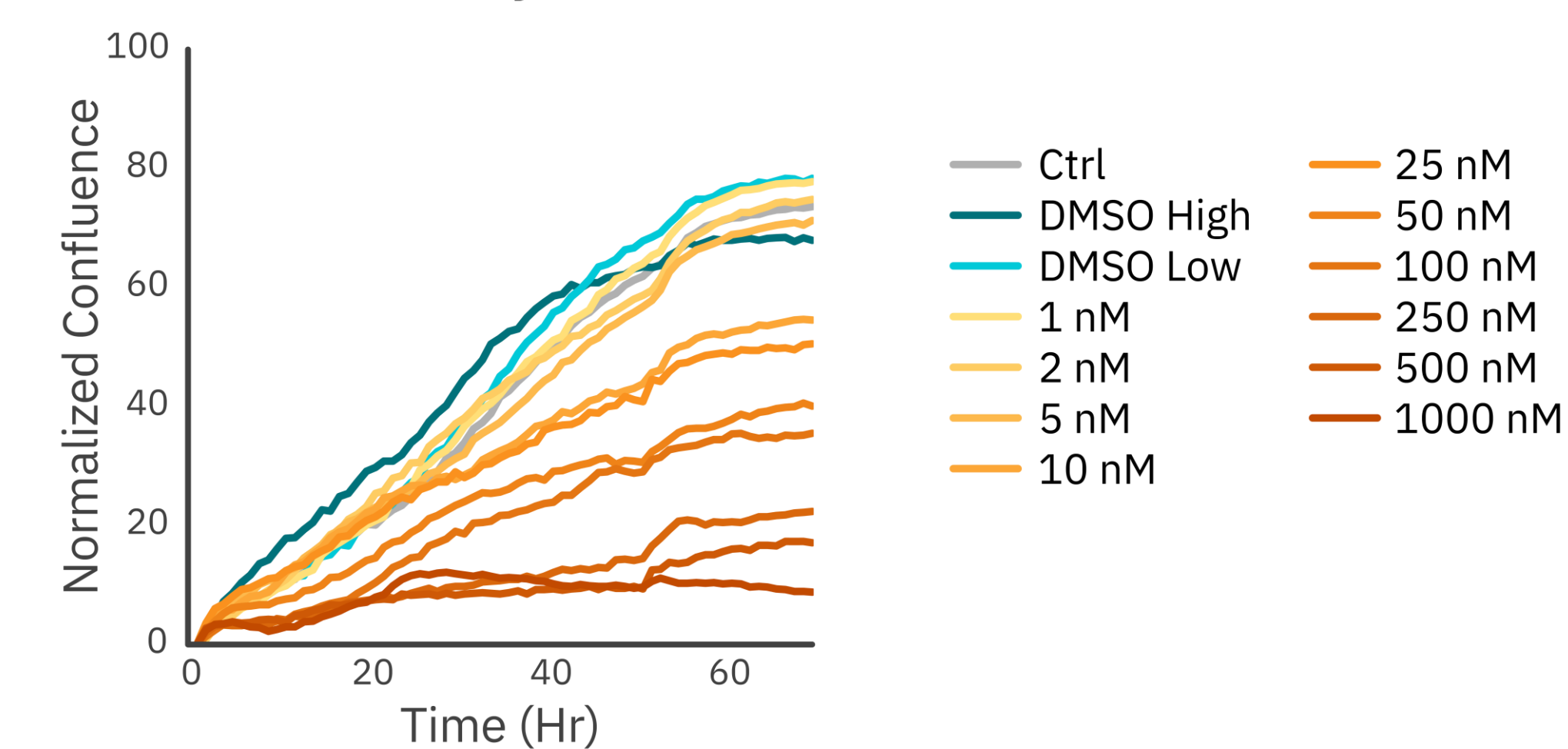
Results

Paclitaxel concentrations of 1, 2, and 5 nM did not affect cell morphology or proliferation (72-77% normalized confluency at 72 h), similar to the untreated control (72% normalized confluency at 72 h). At 10 and 25 nM PX, less severe cell morphology alteration and death was observed compared to 50 and 100 nM PX, with reduced normalized confluency (39-54% at 72 h). PX concentrations of 250, 500, and 1000 nM caused changes in morphology, cell shrinkage, elongated protrusions, and cell death resulting in a significant reduction in normalized confluency (71-89% at 72 h) compared to the untreated control. These results are in alignment with the calculated EC₅₀ of 41.8 nM at 72 h (R² = 0.93).



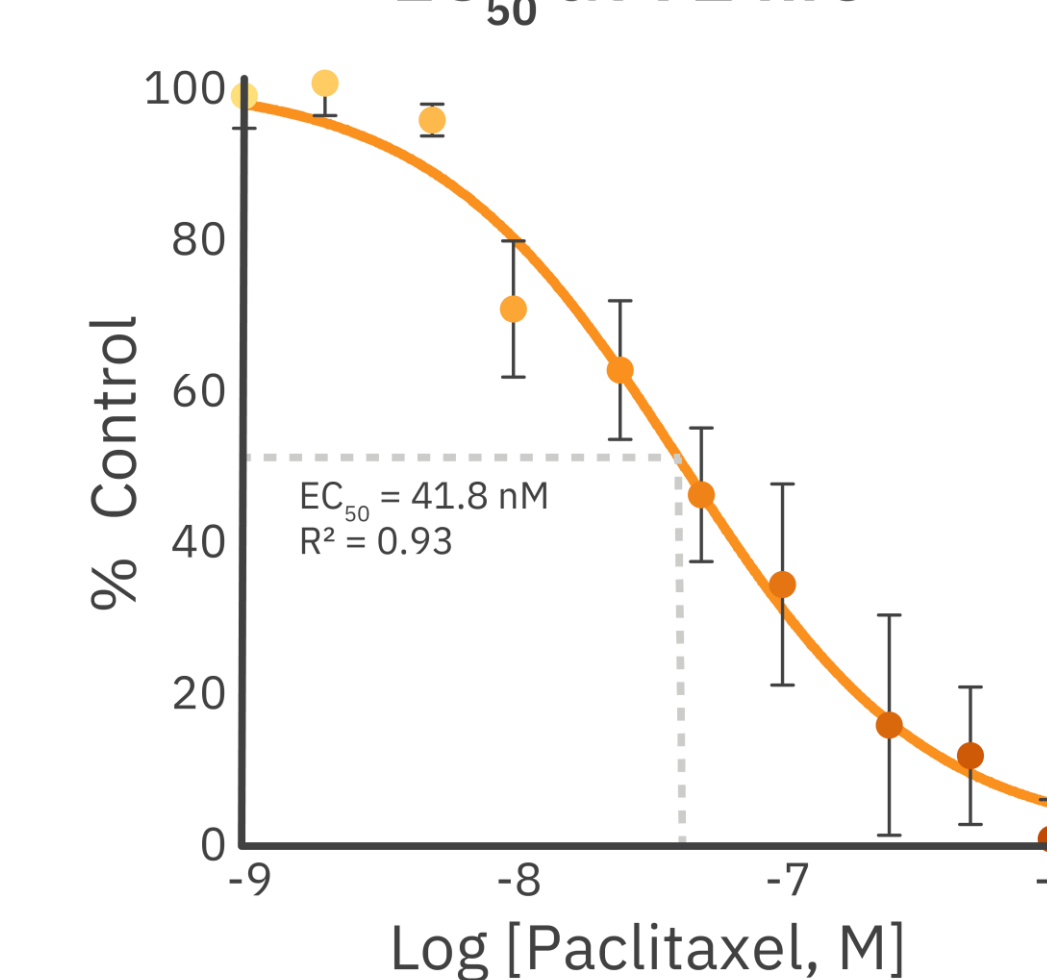
Representative images of C6 cell morphology 20 h after the addition of paclitaxel.

Confluency vs Time



Average normalized confluency (%) of the C6 cells treated with 10 different PX concentrations, n=8 per group.

EC₅₀ at 72 hrs



Half maximal effective concentration (EC₅₀) of PX at 72 h following the treatment.

Conclusion

In this study, it was shown that long-term Paclitaxel treatment with concentrations of 10 nM and higher affects cell viability in a dose dependent manner. Live cell imaging revealed morphological changes preceding cell death, which are not detectable with traditional viability assays such as metabolic activity assays or cell counting.

By using label-free confluency measurements cell viability was monitored in real-time without missing any important timepoints. Furthermore, because of the noninvasiveness of this method, the measured changes in viability were most probably solely caused by addition of Paclitaxel. Overall, label-free confluency measurements offer a valuable non-invasive alternative for long-term cell viability analysis.