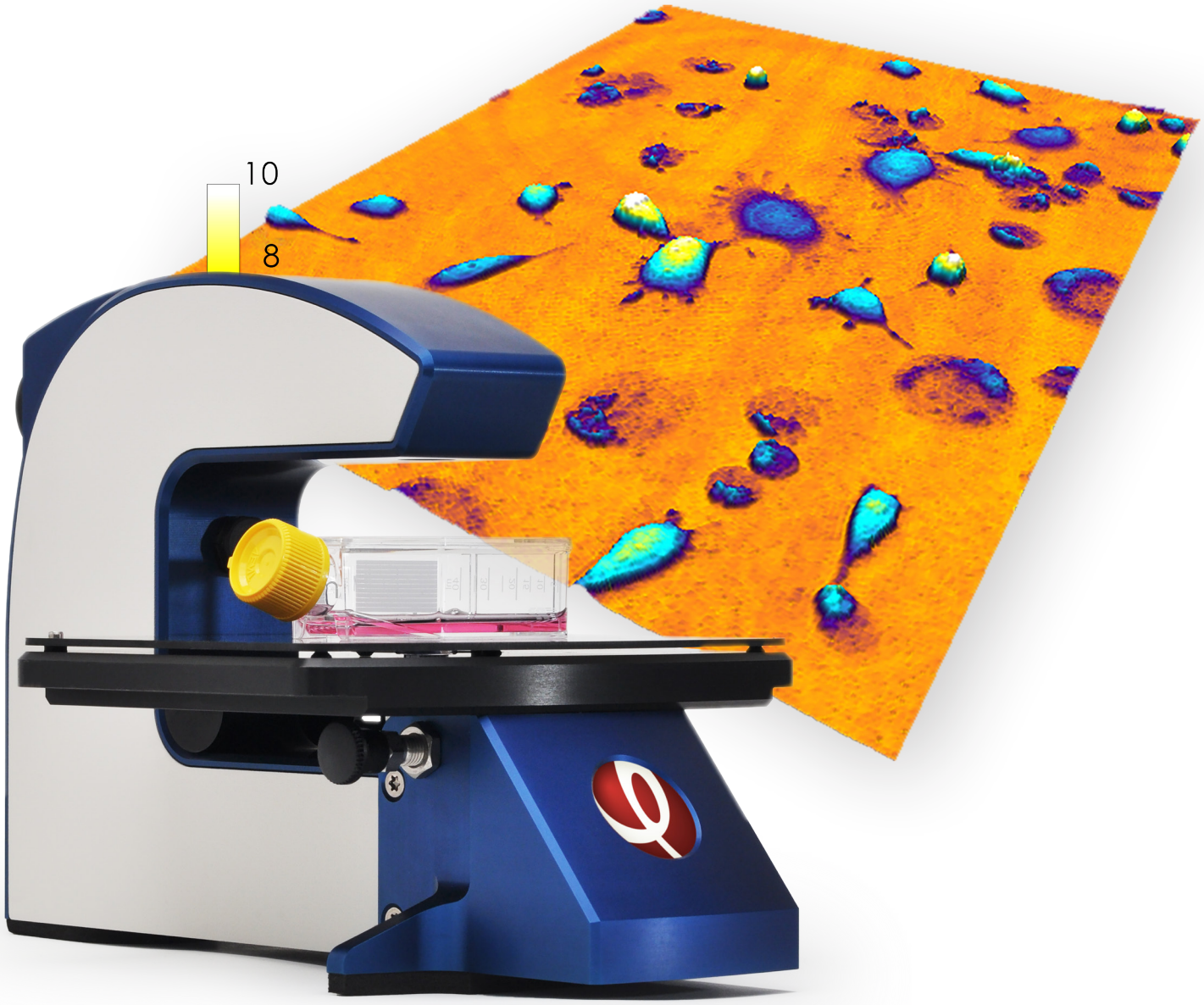
HoloMonitor

application note on

Label-free Toxicology



Holographic Imaging
Phase

Label-Free Toxicology

The HoloMonitor® time-lapse cytometer monitors changes in cell morphology and cell counts non-invasively over time. The analysis includes data for both individual cells and entire populations at the same time, allowing for non-invasive, fast, easy and reliable toxicity studies.

BACKGROUND

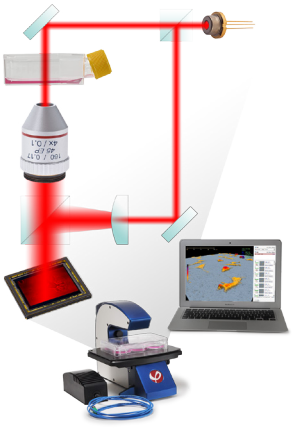
Cell counting, confluence and morphology are useful parameters for toxicity analysis. The HoloMonitor time-lapse cytometer determine cell proliferation, both by cell counting and by confluence (Mölder et al 2008), but also allows for quantitative assessments of cell morphology. Healthy cells are irregular and rather thin, dying cells are small, less irregular and thick, while dead cells are round and thin. These features are the basics for determination of drug effects on cell populations.

Kühn et al. (2013) compared digital holographic microscopy with fluorescence-based toxicology analysis methods and found a good correlation. In addition there was an excellent correlation between cytotoxicity dose response curves obtained by holographic microscopy and known IC50-values for several different toxic compounds. Székács and colleagues (2014) have used the HoloMonitor M4 to measure the cytotoxicity of the herbicide formulation Roundup and its active component Glyphosate, based on cell area changes.

In this application note we demonstrate the usefulness of HoloMonitor for toxicity studies, by showing the effect of the cancer drug etoposide on mouse fibroblast cells (Fig. 1).

HOLOGRAPHIC MICROSCOPY

HoloMonitor create label-free images by dividing red laser light into a reference and an object beam (right). As the object beam passes through the specimen, a phase delay is imprinted on the beam. By subsequently merging the object and the reference beam, this otherwise invisible imprint is recorded by an image sensor. From the recorded hologram, the imprint is numerically reconstructed into a so-called phase image, which is displayed and analyzed (Mölder et al 2008).



METHODS

Mouse fibroblast cells (L929) were seeded with 2×10^5 cells in 5 ml medium in 25 cm^2 flasks. After 24 hours they were treated with etoposide and incubated for 72 hours. Etoposide, which is a topoisomerase inhibitor, was dissolved in DMSO and used at final concentrations of 1, 10 or 100 µM. The same flasks were used throughout the experiment. Every 24 hours, 20 images were captured in each flask at different positions using HoloMonitor. Image analysis with the HoloMonitor software resulted in cell numbers and cell morphology data.

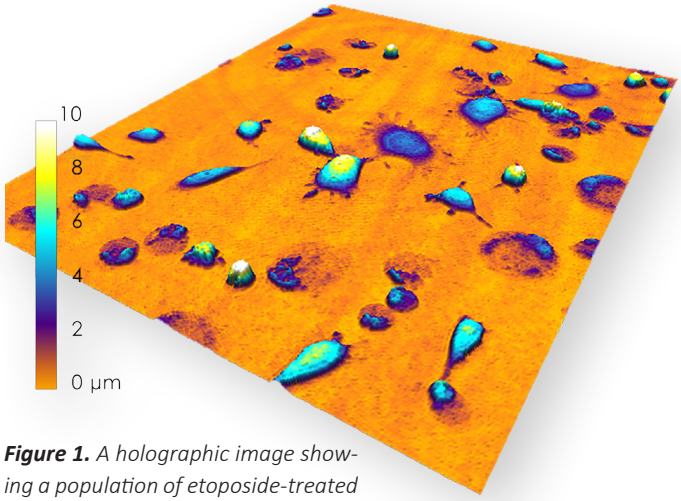


Figure 1. *A holographic image showing a population of etoposide-treated mouse fibroblast cells.*

RESULTS & DISCUSSION

Cell death is a common end point for toxicological studies. However, cell morphology measurements using HoloMonitor make drug effects visible even before cell death occurs. Here we show that etoposide treatment causes cell death. In addition we can follow the process of the etoposide treatment and detect other effects e.g. senescence, seen as enlargements of surviving cells. Images captured days 1-3 of the experiment show that the cell morphology is essentially unchanged in control cells, while the cell morphology of treated cells change over time as the cells become affected by the treatment.

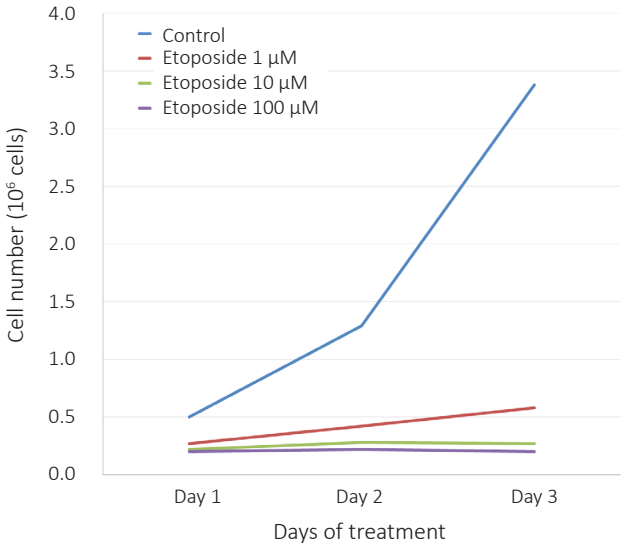


Figure 2. *Growth curves for control and etoposide-treated mouse fibroblast cells based on holographic measurements. n=2 cell flasks.*

Cell proliferation

Control cells proliferated exponentially, while proliferation in cells treated with etoposide was clearly inhibited (Fig. 2). Low concentrations of etoposide allowed cells to continue a slow proliferation, while higher concentrations resulted in total growth inhibition.

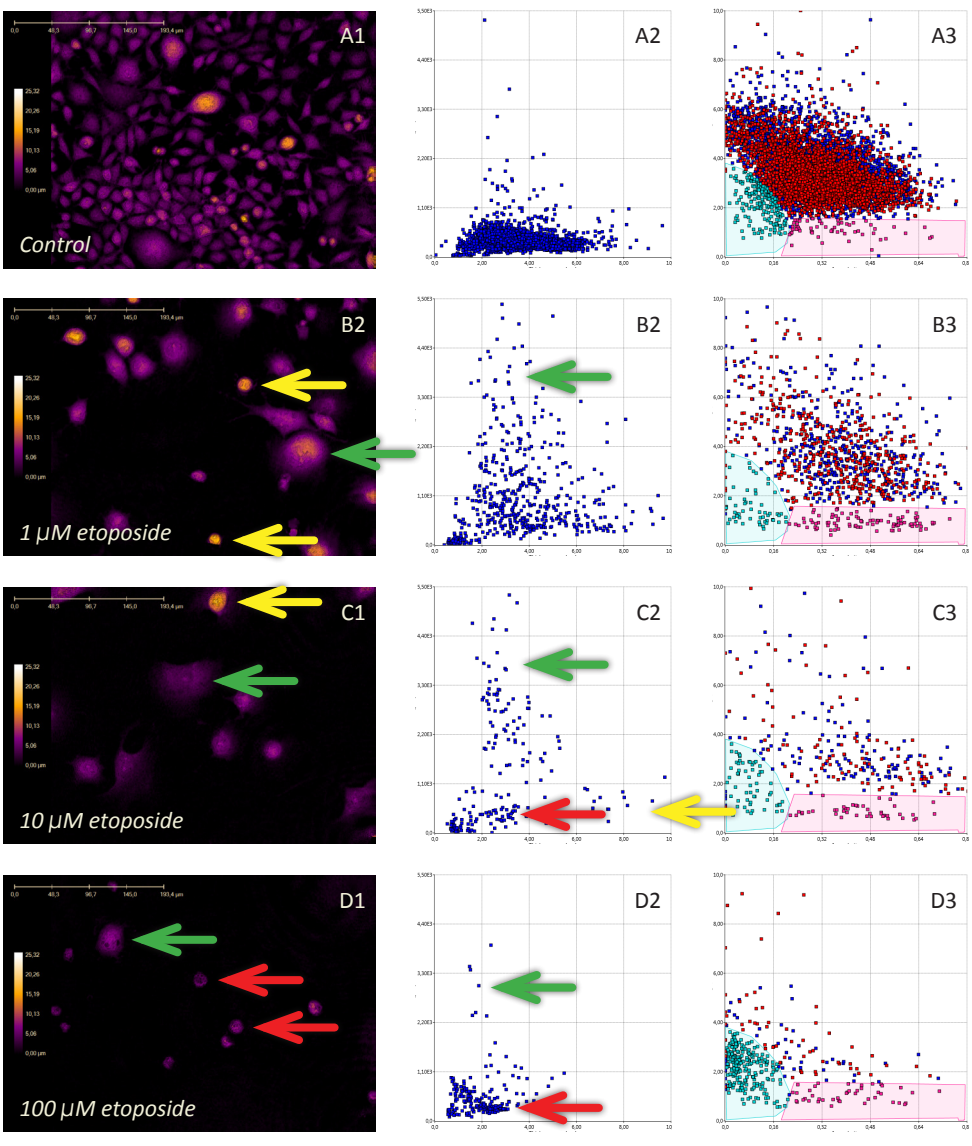


Figure 3. *Image morphologic analysis for mouse fibroblasts after three days of treatment with etoposide. The holographic images (left column) show that etoposide at 1 and 10 µM causes some cells to become large (green arrows) and some to round up and thicken (orange arrow). At 100 µM, cells become thin and small (red arrows). The area/thickness scatterplots (middle column) show separation of dead (red arrows), enlarged (green arrows) and dying cells (orange arrows). The thickness/irregularity scatter plots (right column) show how dead cells (turquoise region), debris (pink region) and living cells can be gated.*

Cell viability

In a diagram with the morphological parameters thickness and irregularity, dead cells cluster together in a separate area in the plot (Fig. 3, right column, turquoise region). This enables determination of the percentages of dead cells in the populations (Table 1).

Table 1*. Fractions of dead cells from day 1 to day 3 of treatment.*

	Day 1	Day 2	Day 3
Control	2%	1%	3%
Etoposide 1µM	3%	2%	5%
Etoposide 10µM	4%	10%	17%
Etoposide 100µM	4%	45%	58%

Cell morphology

A diagram with the morphological parameters area and thickness shows that etoposide treatment at low concentrations resulted in some cells becoming larger than control (Fig. 3, middle column). The large cells did not die, even after three days of treatment. Etoposide has the ability to induce senescence in fibroblasts (Leontieva and Blagosklonny 2010). The senescent cells are much larger than proliferating cells. Therefore the large cells seen in Fig. 3 (B1 and C1) are most likely senescent.

CONCLUSION

Using HoloMonitor, cell proliferation, cell morphology and cell viability can be measured at several time points. Individual cells and whole cell populations are analyzed at the same time. This makes it possible to conveniently evaluate several different toxicological parameters using very few cell samples. The data become more relevant when the same samples are studied, than when separate, replicate cultures are used for different time points and parameters. This contributes to building statistically sound results.

REFERENCES

- Kühn et al, "Label-free cytotoxicity screening assay by digital holographic microscopy", Assay and Drug Developmental Technologies 2013.
- Leontieva and Blagosklonny, "DNA damaging agents and p53 do not cause sencen in quiescent cells, while consecutive re-activation of mTOR is associated with conversion to senescence", Aging 2010.
- Mölder et al, "Non-invasive, label-free cell counting and quantitative analysis of adherent cells using digital holography", J Microscopy 2008.
- Székács et al, "Environmental and Toxicological Impacts of Glyphosate with Its Formulating Adjuvant", International Journal of Biological, Veterinary, Agricultural and Food Engineering 2014.

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