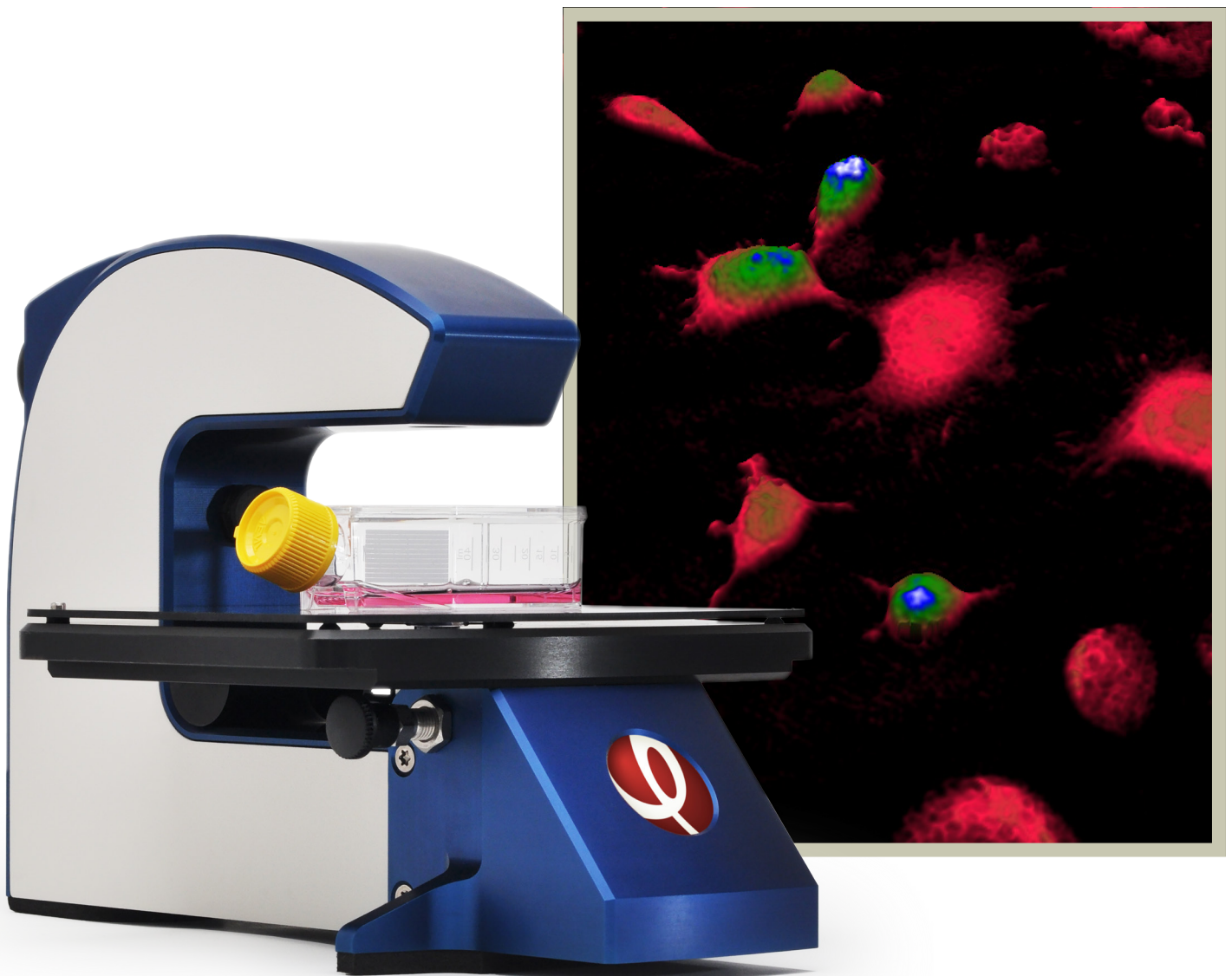


HoloMonitor

application note on

Label-free Cell Viability



Using the HoloMonitor® time-lapse cytometer, cell death and viability can be monitored over time without fluorescent labeling or other treatments. Unlike traditional microscopy, the HoloMonitor provides quantitative data on parameters such as cell area, thickness and volume, which are useful in detection of live/dead cells.

BACKGROUND

Holographic microscopy has successfully been used in cell death/viability studies. The optical cell volume and the optical thickness of a dying cell change over time. Healthy cells are often irregular in shape and thin, dying cells are round and thick while dead cells are round and thin (Fig.1). Khmaladze et al. (2012) and Pavillion et al. (2012) observed a large decrease in optical cell volume during cell death. Living/dead cells have been detected equally well using cell roundness and cell thickness parameters measured with holographic microscopy as with fluorescence-based methods (Kuhn et al. 2013). Kemmler et al. (2007) have used digital holography to study apoptosis in oligodendrocytes.

While volume is an efficient parameter to detect dead cells in homogeneous cell populations, it is not optimal for heterogeneous cell populations due to the overlap in volume between small living cells and large dead cells. HoloMonitor presents alternative parameters to volume, such as optical thickness, area and irregularity.

In this application note we show how HoloMonitor can be used in viability analyses of populations by monitoring and characterizing cell morphology of individual cells during treatment with an apoptosis-inducing drug. The acquired data are then used for further viability analyses of populations. The viability analysis performed using HoloMonitor was compared to manual cell counting using a haemocytometer and trypan blue staining of dead cells.

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).

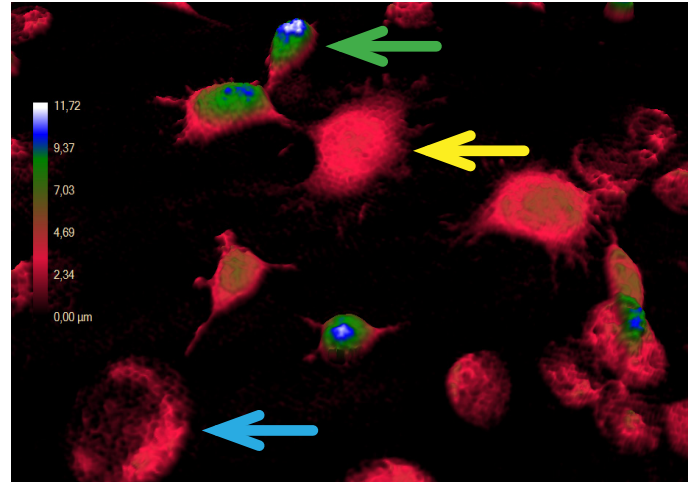


Figure 1. A population of healthy (yellow arrow), dying (green arrow) and dead (blue arrow) L929 cells.

METHODS

For time-lapse cytometry and for the trypan blue cell morphology comparison analysis, L929 mouse fibroblast cells were seeded on single channel IBIDI μ -slide one day before treatment with etoposide (100 μ M).

For the time-lapse cytometry, a time-lapse image sequence was recorded after treatment using HoloMonitor (1 image every 2 minutes for 96 hours). In the recorded time-lapse sequence, the optical volume and thickness of the treated cells were tracked and measured. For the trypan blue cell morphology analysis, cells were imaged with HoloMonitor and with phase contrast microscopy after treatment with trypan blue stain for 10 minutes. For cell counting with the trypan blue exclusion method, cells were seeded in a 6-well plate. One day after seeding, the cytotoxic drug etoposide was added (0.50 or 100 μ M).

Three days after seeding, the cells were imaged and counted using HoloMonitor by recording 30 – 40 random images per well. Thereafter the cells were harvested by trypsinization, taking care to collect both floating and adherent cells, stained and counted according to a standard protocol for trypan blue exclusion.

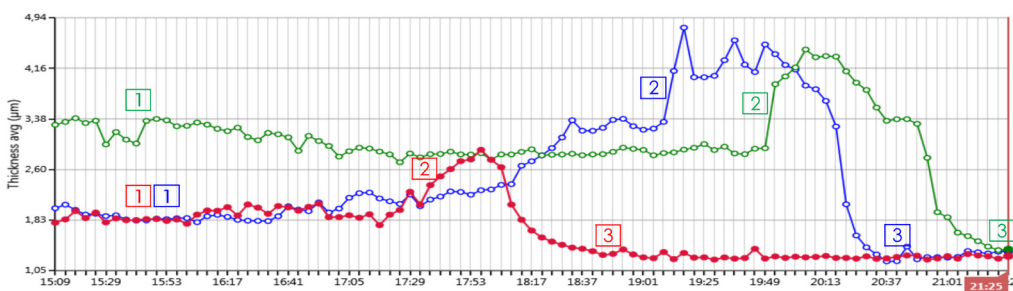


Figure 2. The changes in optical thickness during the death process in three individual etoposide-treated L929 cells as determined by the HoloMonitor. The death process is described with: 1. The cells are healthy. 2. The cells are dying. 3. The cells are dead. Each cell is represented by a colored line. Cells were captured every two minutes.

RESULTS AND DISCUSSION

Using HoloMonitor, we have studied morphological changes during cell death. The observed changes correspond well with what has been reported by Ziegler and Groscurth (2004) and Pavillion et al. (2012).

Time-lapse Cytometry

Cell morphology measured over time is useful in following the death process. The optical cell volume correlated with the death process, and can be used as a viability marker, but the final volume is different for individual cells. As for the average optical thickness, cell death caused first an increase and then a sudden decrease (Fig. 2). The optical thickness was a more consistent viability parameter. Cell irregularity varies greatly over time and between individual cells, but as cells die they become round and this is seen as a clear decrease in irregularity. The results presented in this study are based on cell irregularity and cell thickness.

Holographic detection of dead cells

Living, dying and dead cells can be distinguished using the morphological parameters optical thickness and irregularity. Cells that were optically thin and round (Fig. 3, red region) were found to be identical to cells that were stained with trypan blue (Fig. 3).

Trypan blue cell counts

Trypan blue staining is a well-known method for cell viability determination. The live/dead cell counts based on cell morphology performed with HoloMonitor correspond well with those obtained using the trypan blue exclusion method (Table 1).

To ensure that the trypan blue staining did not affect the HoloMonitor measurements, we compared measurements of the same cells before and after staining. Trypan blue staining was found to have no effect on the measurements (result not shown).

Table 1. Percentage of living and dead cells in etoposide-treated samples of mouse fibroblast cells. Each sample was counted with both methods, and results are given for each of two samples per treatment.

	Trypan blue cell count	HoloMonitor cell count
Control	97/96%	99/98%
50 μ M	45/72%	48/72%
100 μ M	73/72%	57/78%

CONCLUSION

The HoloMonitor is well suited for viability studies. Holographic microscopy is non-invasive and non-phototoxic in contrast to other methods. Using time-lapse imaging, cultured cells can be monitored over several days. The time-lapse recordings contain vast amounts of information about each individual cell, allowing several different morphological parameters to be used for viability analysis. For L929 cells, the parameters thickness and irregularity are well suited to identify dead cells. In contrast to e.g. flow cytometry all resulting data can be back-tracked to each individual cell at each specific time-point.

REFERENCES

- Kemmler et al, "Noninvasive time-dependent cytometry monitoring by digital holography", Biomed Opt (2007)
- Khmaladze et al, "Cell volume changes during apoptosis monitored in real-time using digital holographic microscopy", J. of Structural Biology (2012)
- Kuhn et al, "Label-free cytotoxicity screening assay by digital holographic microscopy", Assay and Drug Development Technologies (2013)
- Mölder et al, "Non-invasive, label-free cell counting and quantitative analysis of adherent cells using digital holography", J. of Microscopy (2008)
- Pavillon et al, "Early cell death detection with digital holographic microscopy", PLOS one (2012)
- Ziegler and Groscurth, Morphological features of cell death, Physiology (2004)

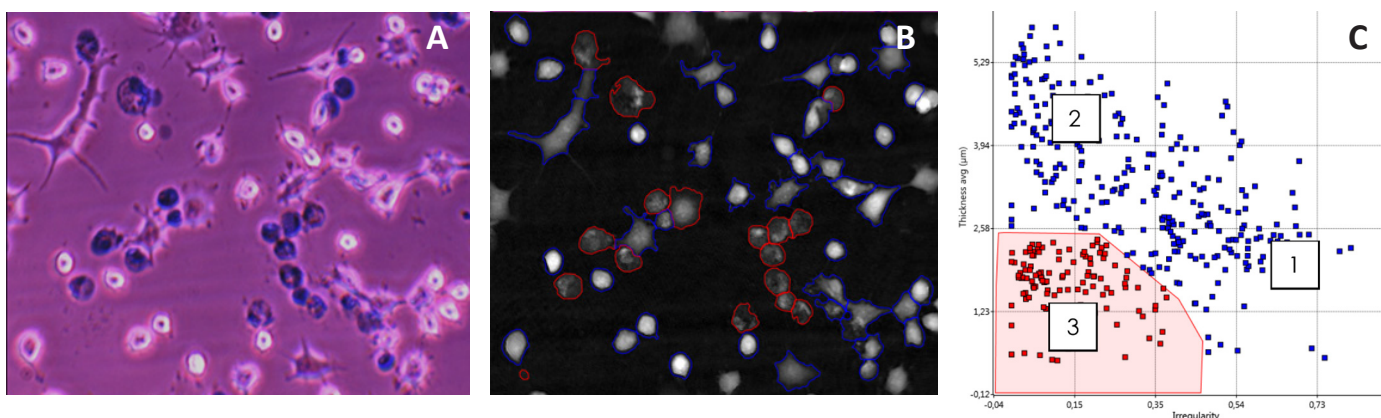


Figure 3. A: phase contrast image of etoposide-treated cells stained with trypan blue. B: The same cells imaged with HoloMonitor. C: A scatter plot based on 10 HoloMonitor images, displaying cell thickness and irregularity. Healthy cells, which are irregular and thin, are located at position 1 in the scatter plot. Dying cells contract, thus becoming thicker and round, are found at position 2. Dead cells are thin and round and are found in the red region (position 3). In B, dead cells are outlined in red. The healthy and dying cells, outside of the red region, are outlined in blue. By comparing A and B, it can be seen that label-free identification of dead cells corresponds well to the trypan blue identification method.