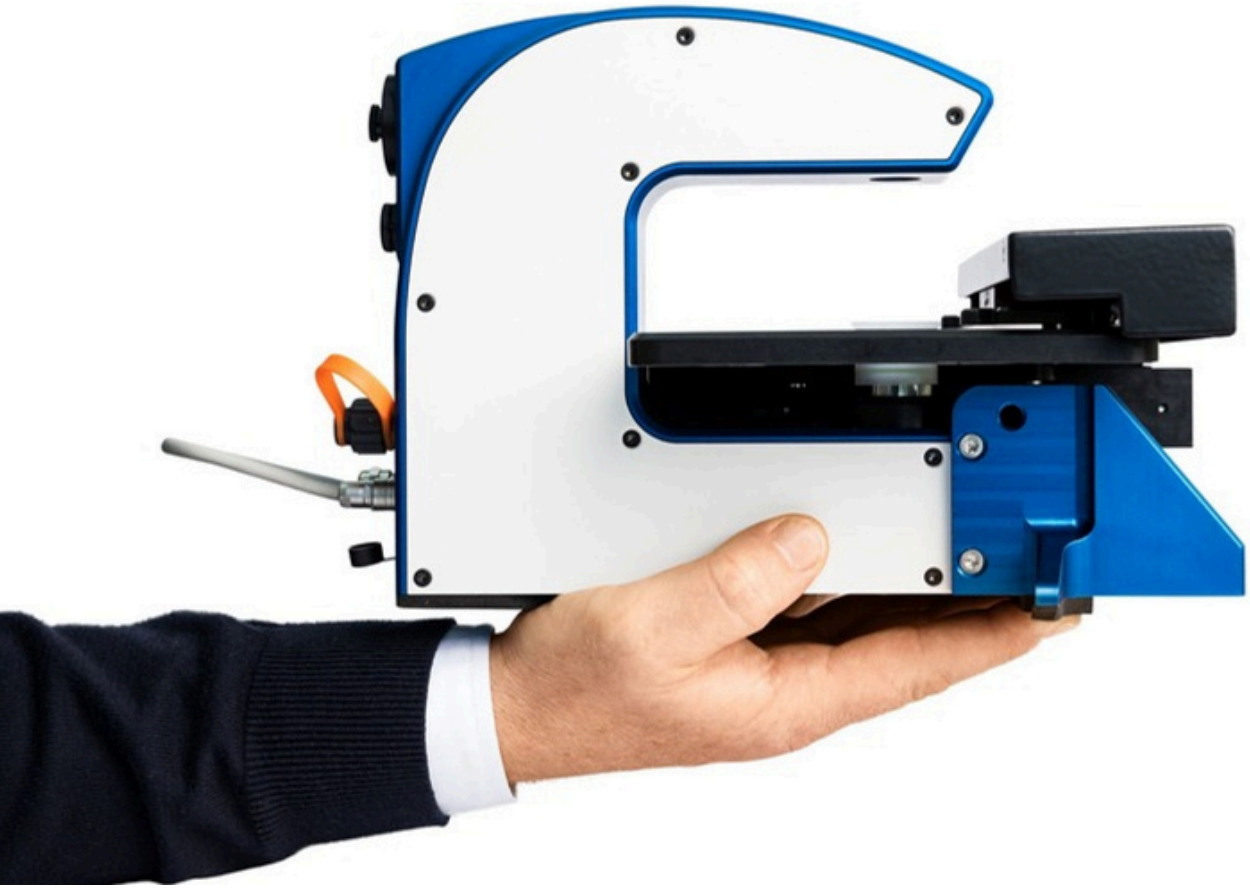
APPLICATION GUIDE



The HoloMonitor technology makes cells visible without labels or stains. Together with the guiding App Suite software a range of cellular parameters can be explored in-real time with minimal user effort. This guide explains how you can easily get publication-ready kinetic results and use single cell tracking for analysis of individual cells and cell populations.

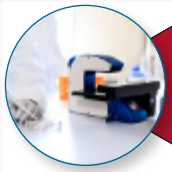
You will learn how to get the whole picture of *in vitro* drug effects, understand how cell population behavior changes and explore details of single cells — all in one experiment.

CELL & USER FRIENDLY

The HoloMonitor® time-lapse cytometer is used by scientists all over the world to study the life cycle and behavior of living — or dying — cells under physiologically optimal conditions inside the cell incubator.

Setup of an experiment is easy and straight forward. The technology employed, holographic microscopy, is completely non-invasive and makes cell visible without label or stains. HoloMonitor operates inside the cell incubator, or hypoxia chamber, ensuring cells are studied at optimized conditions.

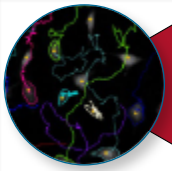
HoloMonitor is thus cell-friendly, fast, and easy to use. It offers unique imaging capabilities for live-cell kinetic investigations of both single cells and cell populations.



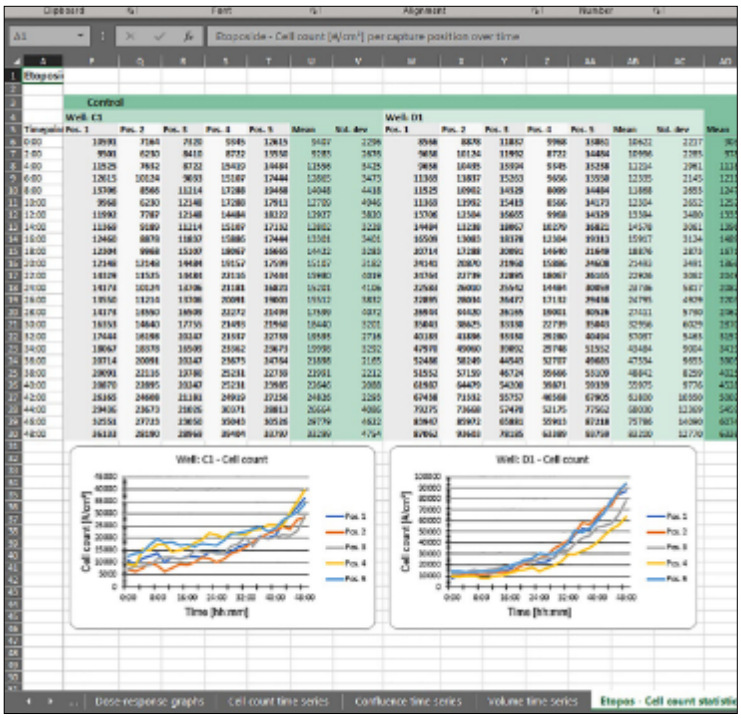
ALL LABEL-FREE
easy and straight forward setup

INCUBATOR COMPATIBLE
cells are studied in their own environment

GUIDING SOFTWARE
designed for optimal cell and user friendliness



ONE EXPERIMENT
multiple results



Real-time result presentation

Using HoloMonitor and the App Suite software you can easily capture images, videos and quantitative data.

The software automatically extracts selected cellular parameters. For each application relevant data is extracted and presented in real-time.

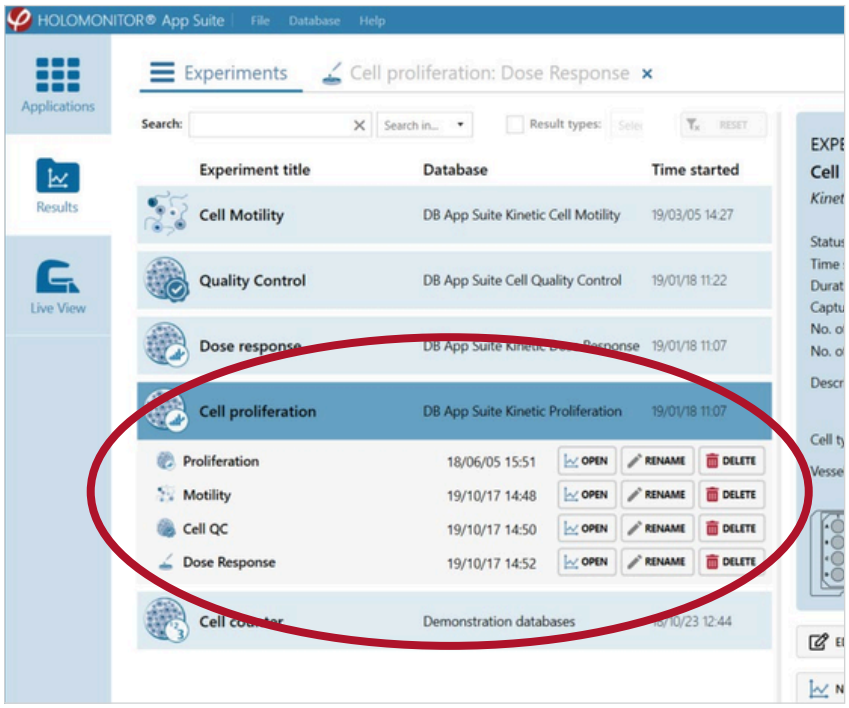
With just one click you can export all data to Excel for deeper understanding and flexible result presentation.

UNDERSTAND DRUG EFFECTS — IN JUST ONE EXPERIMENT

From just a single time-lapse experiment you can learn how your drug affects your cells. HoloMonitor acquires a series of cell images at regular time intervals, set by the user. The same cells are monitored over time to analyze the dynamics of various cellular events. In addition to identifying each individual cell, HoloMonitor provides information for the study of more than 30 morphological parameters.

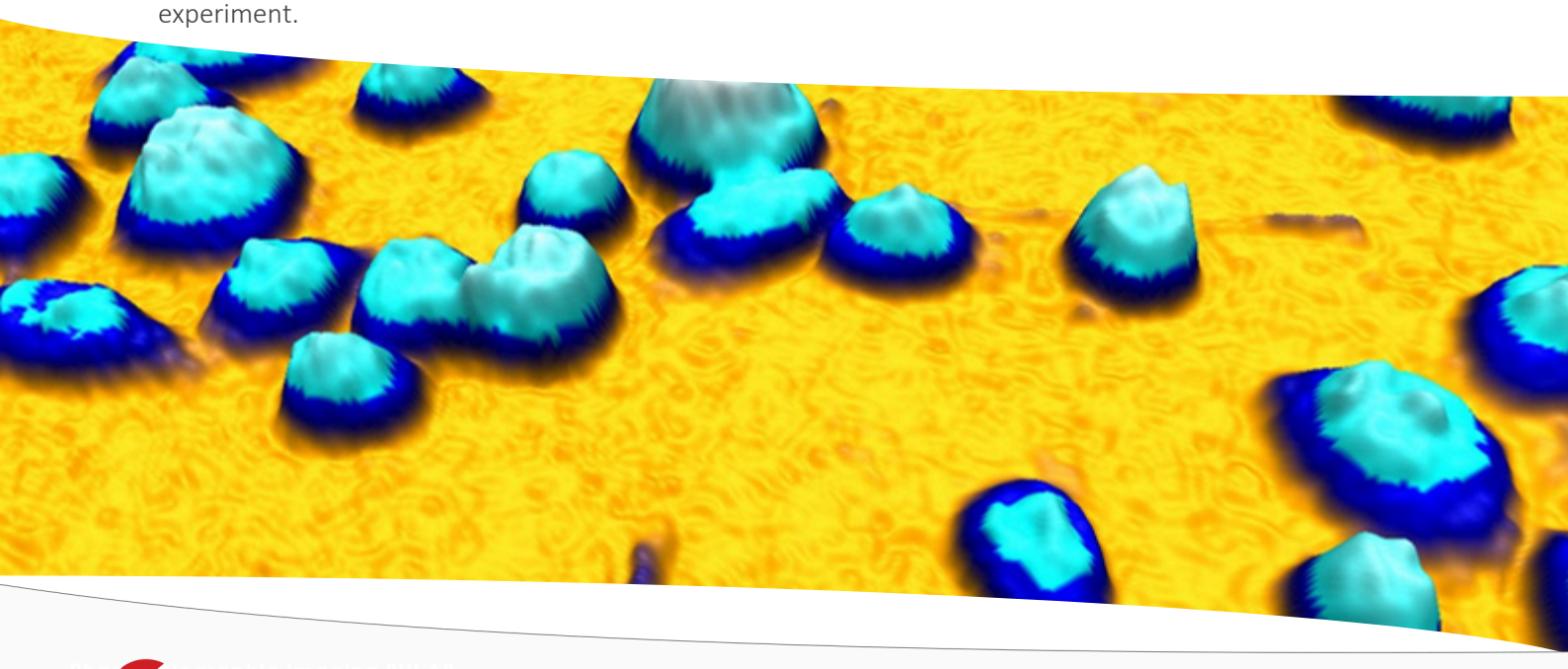
Non-destructive — saving time, effort and cells

The HoloMonitor imaging technology do not affect the cells in any way. Since no labels are used, the cells and/or the supernatant can be saved for further studies using other methods after imaging. HoloMonitor thus saves both time, effort and cells.



One experiment — multiple results

From the recorded time-lapse images, the HoloMonitor App Suite software automatically extracts and analyzes kinetic live cell population data based on individual cell data. When preferred, individual cell data — such as cell count, cell morphology and spatial cell tracking of individual cells — can all be analyzed from the same experiment.



LIVE CELL IMAGING & ANALYSIS ASSAYS

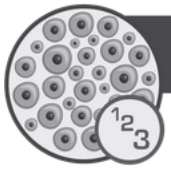
The HoloMonitor label-free live cell imaging system offers a powerful and unique portfolio of cell biological applications. All applications are aimed for adherent cells, apart from the Cell Counter that offers automatic counting of cells in suspension.

Guided end-point assays

—Quick assessment of cell count and cell culture quality

Cell QC Assay

- Cell culture quality control



Cell Counter

- Cell count of cells in suspension

Guided kinetic assays

—Publication-ready kinetic results with minimal effort

Dose Response Assay

- Dose response curves at all time points

Cell Proliferation Assay

- Cell count and confluence over time

Cell Motility Assay

- Cell speed and accumulated distance

Wound Healing Assay

- Kinetic and automatic gap closure data

In-depth analysis

—Single cell tracking for analysis of individual cell and/or cell populations

Spatial Cell Tracking

- Follow single cells

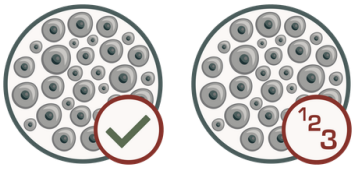
Kinetic Morphology

- +30 morphological parameters

GUIDED END-POINT ASSAYS

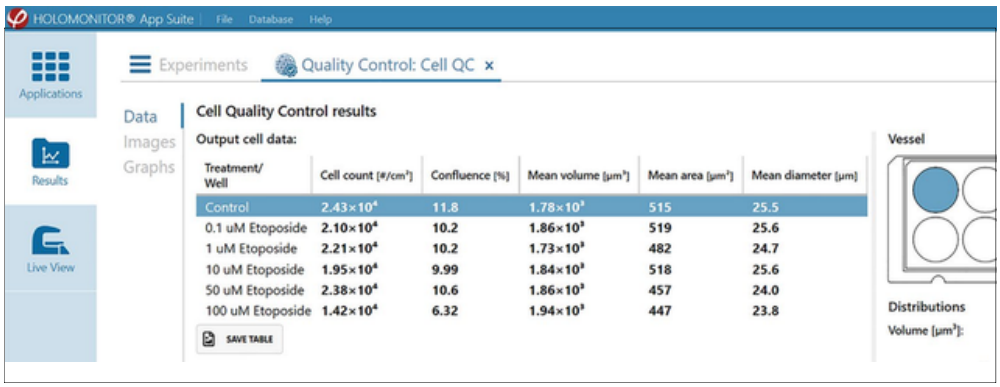
Cell QC Assay and Cell Counter

—Quick assessment of cell count and cell quality control



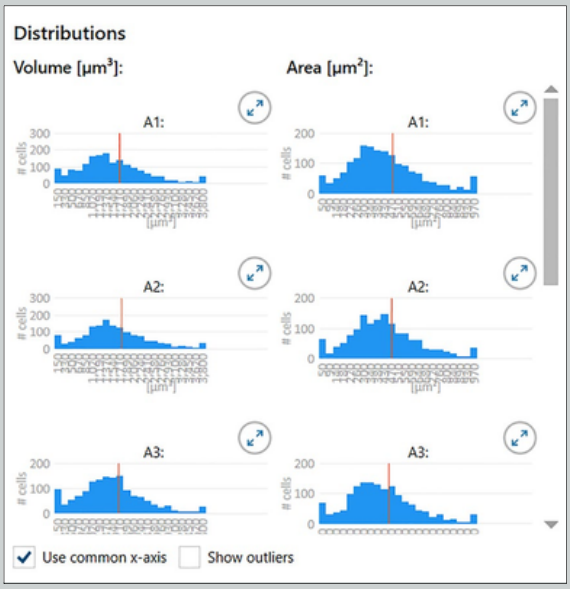
Cell quality control (QC) is fundamental to ensure the quality and reproducibility of cell-based experi ments.

The Cell Counter Assay offers automatic counting of cells in suspension, saving time and avoiding user bias, while the Cell QC Assay is designed to evaluate adherent cell culture integrity before the experiment. In addition, both assays provide key information on cell morphology.

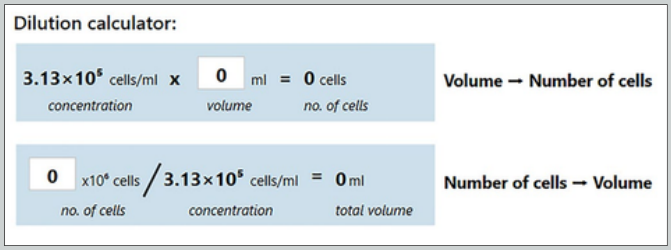


Treatment/ Well	Cell count [#/cm²]	Confluence [%]	Mean volume [µm³]	Mean area [µm²]	Mean diameter [µm]
Control	2.43×10^4	11.8	1.78×10^3	515	25.5
0.1 uM Etoposide	2.10×10^4	10.2	1.86×10^3	519	25.6
1 uM Etoposide	2.21×10^4	10.2	1.73×10^3	482	24.7
10 uM Etoposide	1.95×10^4	9.99	1.84×10^3	518	25.6
50 uM Etoposide	2.38×10^4	10.6	1.86×10^3	457	24.0
100 uM Etoposide	1.42×10^4	6.32	1.94×10^3	447	23.8

End point results per well provided by the Cell QC assay.



Distribution graphs showing basic cell morphology are part of the automatic result presentation in the Cell Counter and Cell QC assay.



The Cell Counter Assay also provides a convenient dilution calculator to ensure correct cell numbers for seeding.

Assay benefits

- Ensure correct cell count for seeding
- Detect undesired changes of your cell culture
- Ensure reproducibility of experiments
- Save time and avoid user bias

Phase Holographic Imaging PHI AB

A Brand of Altium

GUIDED KINETIC ASSAYS

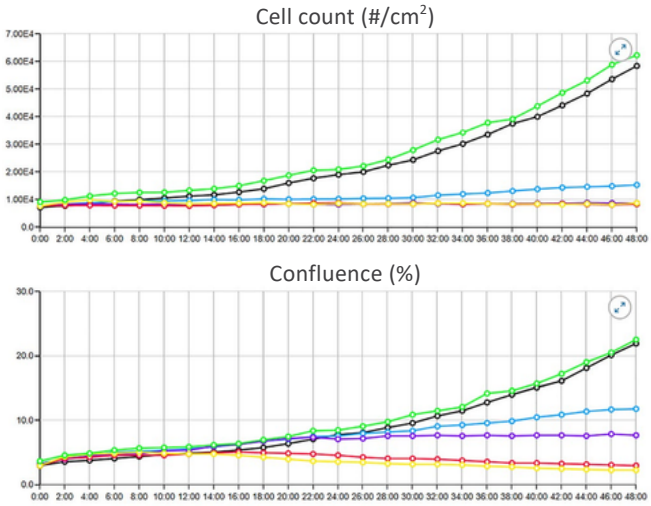
Cell Proliferation Assay

—Automatic presentation of kinetic cell proliferation

Cell proliferation assays are widely applied in life science to understand the growth pattern of cultured cells and to assess the *in vitro* safety and efficacy of drugs over time.

Traditional methods are end-point assays that often assess cell proliferation indirectly or are based on cell confluence measurements only.

HoloMonitor offers a convenient assay that automatically presents kinetic cell proliferation data. Cell proliferation is directly determined by assessing both cell count and cell confluence during the whole experiment.



Kinetic Proliferation Results: control (green line) and various drug concentrations

Assay benefits

- Robust and kinetic cell proliferation assessment with high temporal resolution
- Individual cells are automatically identified and counted by the software — results are based on direct and continuous cell counting

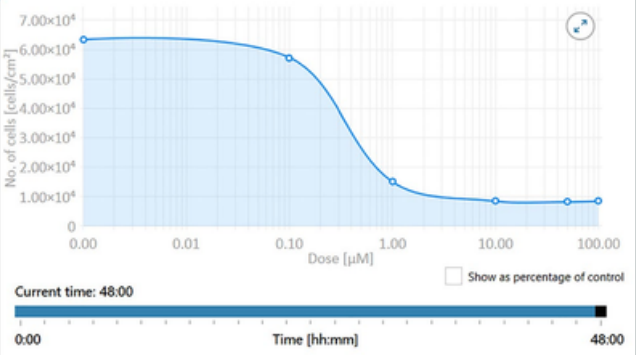
Kinetic Dose Response Assay

—Complete dose response analysis in just one experiment

Understanding the dose response relationship of drugs over time is essential when developing effective cancers treatments or new drugs. The dose response curve of adherent cells is a vital tool in drug screening and pre-clinical research.

The dose response relationship allows drug efficiency and potency to be determined *in vitro* to provide guidance for future studies.

Dose response graph

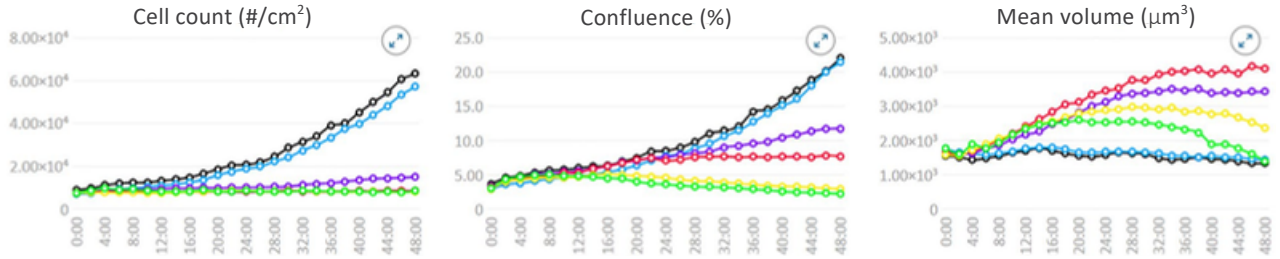


The HoloMonitor Kinetic Dose Response Assay automatically provides interactive *in vitro* dose response curves at any time point.

Dose response results for each time point is achieved from just one single experiment. The slider is used to select time point to be displayed.

Additionally, knowledge of drug effects on cell population morphology facilitates advances in drug development and future disease treatments.

Growth and morphology time-series

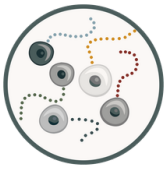


Assay benefits

- Save time and effort — all time points and various concentrations in one setup
- Kinetics of drug effects and toxicity patterns
- Easily compare effects from various compounds and concentrations against controls

Kinetic Cell Motility

—Explore how various drugs and conditions affects motility



Cell motility assays are essential in cancer research and many other fields in cell biology. When tumors metastasize, cancer cells travel through the body and reach a new location where another tumor can develop. Thus, understanding how different treatment options affect cell motility and migration is essential, especially when exploring the effects of new treatment options in the fight against cancer.

LABEL-FREE CELL IDENTIFICATION
Cells are imaged and identified without labels or stains

INCUBATOR COMPATIBLE
HoloMonitor operates in the incubator ensuring cell integrity

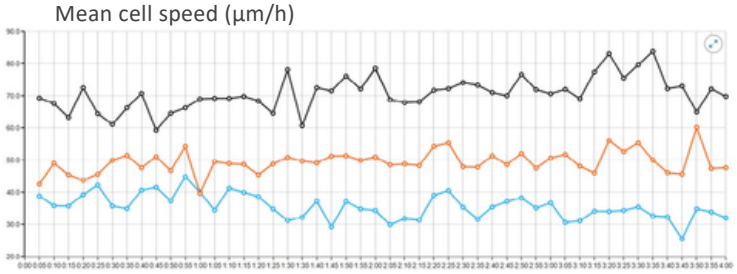
KINETICS & CELL-FRIENDLINESS
The unharmed cells can be studied over time, as long and often as is needed to get relevant motility and migration data

HoloMonitor is ideal for motility and migration studies due to the cell-friendly technology and ability to study individual cells over time inside the cell incubator.

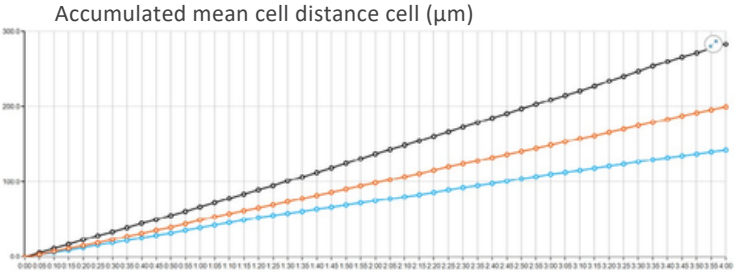
The HoloMonitor Motility Assay is designed to explore the average motility of cells in a population and is ideal for studies investigating how various treatments influence cell motility over time.

The assay automatically analyzes and presents cell motility data in terms of speed kinetics, i.e. average cell speed over time and accumulated mean distance, at each time point.
The results are presented immediately, as the software analyzes the data in real time and while HoloMonitor captures images of the cells inside the cell incubator.

Mean cell speed (µm/h)



Accumulated mean cell distance cell (µm)

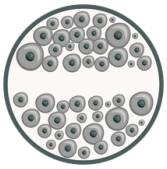


Assay benefits and opportunities

- Detection of differences in cell motility caused by the investigated drugs or environmental conditions.
- Using the same images and data, further motility and migration studies of single cells, or subpopulations of cells, can be performed using cell tracking.

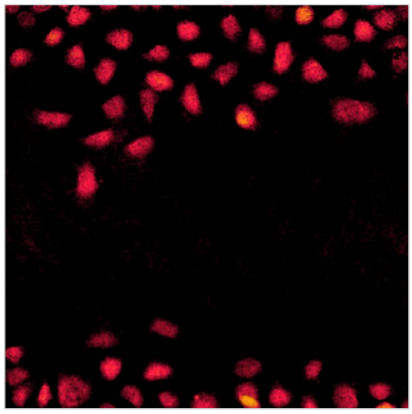
Wound Healing Assay

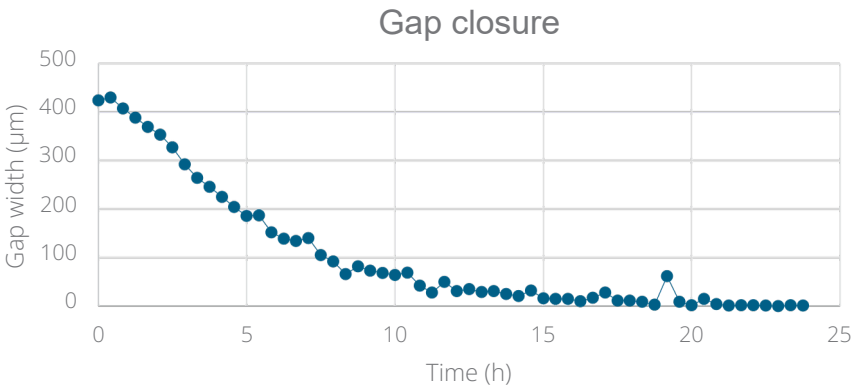
—Smooth,reproducible and cell-friendly with minimum manual effort



Wound healing assays, also known as scratch assays, are widely used to study the coordinated movements of cells, and to measure cell migration across a wound field gap.

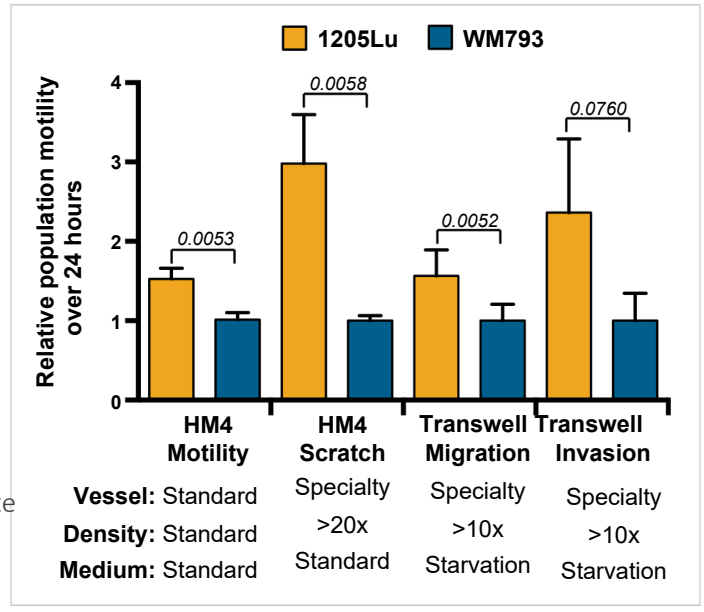
The HoloMonitor Wound Healing Assay is optimized to easily provide kinetic and automatic results, including gap closure and cell front velocity data. In addition, videos showing the gap closure can easily be generated.





To get single cell data and movement patterns, cells of interest can be tracked using cell tracking. Detailed, non-biased data on migration into the wound area is then easily achieved.

In Zhang et al 2018* the HoloMonitor Wound Healing Assay was evaluated and compared to other assays. It was found to be the most tractable and automated method.



*Zhang et al., Evaluation of Holographic Imaging Cytometer Holo-Monitor M4® Motility Applications, Cytometry Part A (2018)

Assay benefits

- Cell front velocity
- Time-lapse videos showing how cells migrate into the gap
- Automatic and kinetic calculation of gap closure over time

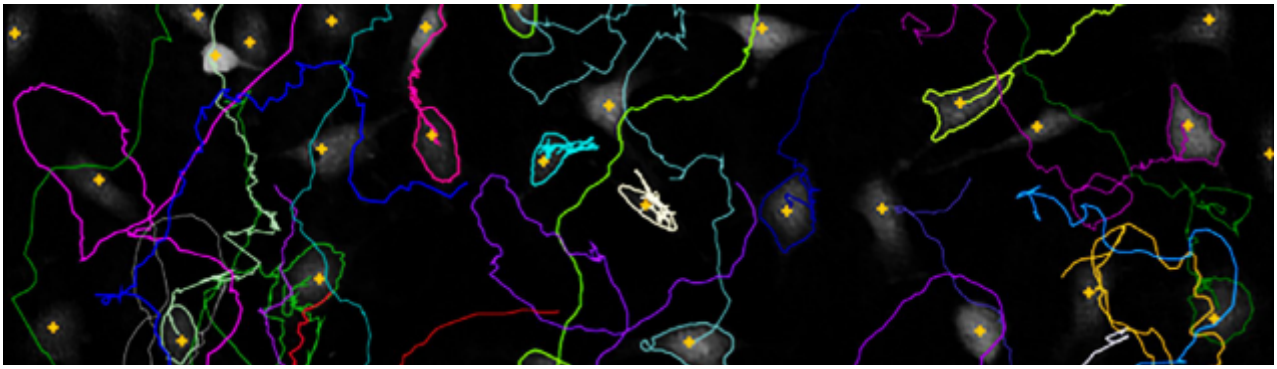
IN-DEPTH ANALYSIS — EARLY AND SENSITIVE DETECTION OF IN VITRO DRUG EFFECTS

The single cell tracking enables in-depth analysis of both individual cells and entire cell populations at the same time.

The possibility to track single cells is the most advanced and powerful feature of HoloMonitor. It allows monitoring and analysis of cell movement and cell morphology of selected individual cells. Details and changes over time are provided for each cell included in the analysis, and in mean values for the selected population.

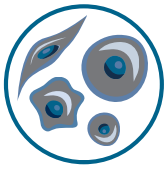
Spatial Cell Tracking

Selecting single cells for detailed motility and migration studies can provide important information on cell speed and direction. Moreover, subpopulations of cells behaving different than the population as a whole can be identified and analyzed separately.



Cell Morphology — A Multi-Parameter Toolbox

Kinetic cell morphology data provides key information on cell health and can be very useful for toxicity analysis, since cell morphology changes often precede cell damage and cell death. HoloMonitor reveals information on more than 30 morphological parameters, which can be useful as early and sensitive markers for drug effects on cells.



Area | Thickness | Volume | Texture | Irregularity

KEY REFERENCES

1. *Quantitative phase imaging for label-free analysis of cancer cells — focus on digital holographic microscopy,* Z. El-Schich et al., Appl. Sci. (2018)

2. *Evaluation of holographic imaging cytometer HoloMonitor M4® motility applications,* Y. Zhang and R. L. Judson, Cytometry Part A (2018)

3. *Moving into a new dimension: Tracking migrating cells with digital holographic cytometry in 3D,* A. Gjörloff Wingren, Cytometry Part A (2018)

4. *Comparative cell biological study of in vitro antitumor and antimetastatic activity on melanoma cells of GnRH-III-containing conjugates modified with short-chain fatty acids,* E. Lajkó et al., J. Org. Chem (2018)

5. *TRPM2 modulates neutrophil attraction to murine tumor cells by regulating CXCL2 expression,* M. Gershkovitz et al., Cancer Immunology, Immunotherapy (2018)

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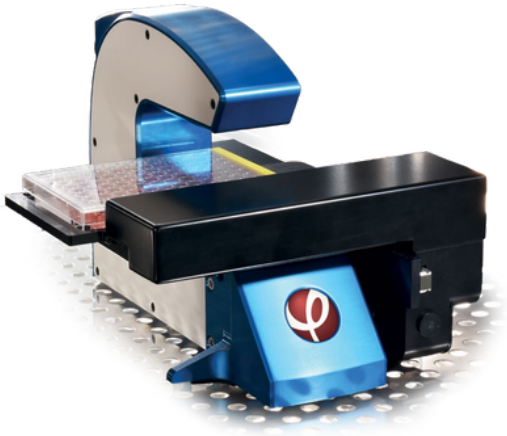
7. *Label-free High Temporal Resolution Assessment of Cell Proliferation Using Digital Holographic Microscopy,* B. Janicke, A. Kårsnäs, P. Egelberg and K. Alm, Cytometry Part A (2017)

8. *Cells and Holograms — Holograms and Digital Holographic Microscopy as a Tool to Study the Morphology of Living Cells,* K. Alm, Z. El-Schich, M. Falck Miniotis, A. Gjörloff Wingren, B. Janicke and S. Oredsson, Holography — Basic Principles and Contemporary Applications (2013)

"Every laboratory working with cell cultures should have a HoloMonitor"

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