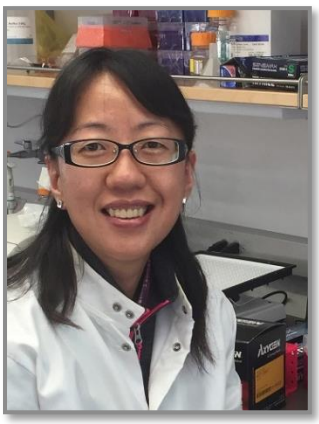
# Application Note

on

# Holographic Imaging Cytometer HoloMonitor M4® Motility Applications

University of California San Francisco

## FEATURED SCIENTIST

*"Both HoloMonitor Track Cell module and the Wound Healing Assay were found to be well-correlated with established standards, yielded reproducible results, and at the same time offered distinct advantages over the transwell assays, including the use of standard culture conditions, cell densities and vessels and the ability to use the assayed cells for other purposes upon completion"*

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# Evaluation of HoloMonitor® M4 Cell Motility Applications as compared to Standard Methods

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## ABSTRACT

Digital holographic cytometry (DHC) allows for long term, label free, non-phototoxic imaging of mammalian cells. When using DHC, individual cells can be monitored over time, and simultaneously population data is gained. This makes it possible to observe sub populations that behave differently than the bulk of cells. Standard methods for analyzing cell motility have limitations as they measure only population averages and deviate from standard routine culturing conditions. These deviations may induce artefacts in the results.

HoloMonitor® M4 DHC platform (Phase Holographic Imaging AB, Lund, Sweden) includes several commercially available cell motility applications. In this study the software modules for cell tracking, wound healing and population motility, were evaluated based on outcome and reproducibility, and results compared to the standard methods, Boyden transwell assays.

## BACKGROUND

Cell motility assays are essential in cancer research and many other fields in cell biology. The widespread standard *in vitro* methods measure relative motilities of cell populations. Among them are the Boyden transwell migration and invasion assays, and the wound healing/scratch assays. These assays measure population averages only and require culturing conditions that differ from normal conditions. Serum starvation prior to the experiment is one example of treatment that can interfere with the results. DHC, on the other hand, provides motility data for each individual cell, which can be essential when studying heterogeneous *ex vivo* cell populations, without scarifying the integrity of the cell culture.

We have previously published results (Zhang and Judson 2018) evaluating HoloMonitor M4 cell tracking and wound healing assays regarding outcome and reproducibility. Results were analyzed using the Hstudio software and compared with the Boyden transwell assays. In this application note we also include previously unpublished results obtained using the new proprietary software App Suite Kinetic Motility Assay.

## HOLOGRAPHIC MICROSCOPY

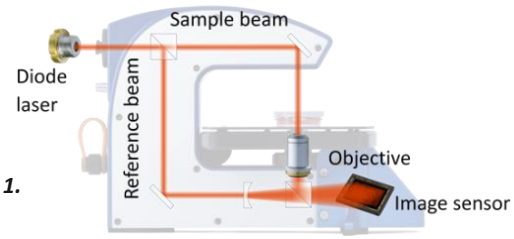


***Figure 1.*** *HoloMonitor creates label-free images by dividing red laser light into a reference and an object beam (fig.1). As the object beam passes through the specimen, a phase delay is imprinted on the beam. After subsequently merging the object and the reference beam, this otherwise invisible 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

Two human melanoma cell lines were used, the non-metastatic (less motile) WM793 and the metastatic (more motile) 1205Lu line. For individual cell tracking with DHC, the cells were seeded in standard 6-well plates in standard culture conditions with 11 000 cells/cm$^2$ and left to attach for 24 hours. Thereafter they were imaged every hour for 24 hours. The images were computationally segmented and individual cells were identified and tracked through the time-lapse. Cells that could not be tracked through the entire time-lapse were excluded from the analysis. The motility of each cell at each time point, speed and total motility (the sum of all motilities over the duration of the time span) were calculated. Also, the number of cells needed to obtain statistical significance was evaluated. For the wound healing assay, 50 000 cells were seeded in each ibidi insert chamber in an ibidi 35 mm dish. The resulting gap was imaged for 24 h and thereafter analyzed with the Hstudio wound healing assay.

For the transwell migration and invasion assays, the cells were serum starved for 24 hours, dissociated and seeded at a density of 120 000 cells/cm$^2$ on inserts with membranes with 8 µm pores. For migration assay, the membranes were tissue culture treated and for invasion assay, they were coated with base membrane extract. After 24 hours, the total number of cells that had migrated was calculated using Calcein AM labelling. For more details, please refer to Zhang and Judson 2018.

## RESULTS AND DISCUSSION

Several approaches to study cell motility were performed to obtain results on population level as well as individual cell level. The relative values on population motility from DHC and the standard methods Boyden transwell migration and invasion assays were compared.

Representative holographic images with corresponding tracking images are shown in figure 2. The identification and tracking are basics for all further analysis.

Tracking 50 or more cells was enough to obtain high reproducibility of the results (fig.3).

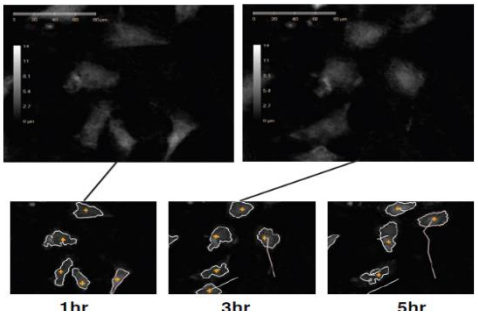


***Figure 2.*** *representative DHC images showing 5 cells. The cells are identified and tracked for 1,3 or 5 hours. The trails show how the cells have moved over time.*

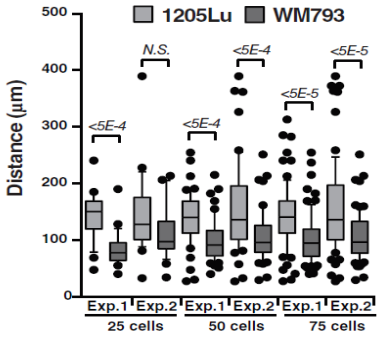


***Figure 3.*** *Single cell tracking data from two 18h time-lapses captured with DHC.*

When individual cells were tracked, findings of rare hyper motile cells in both cell lines (fig. 4) added extra information about the cells that could not have been detected using the classical reference methods.

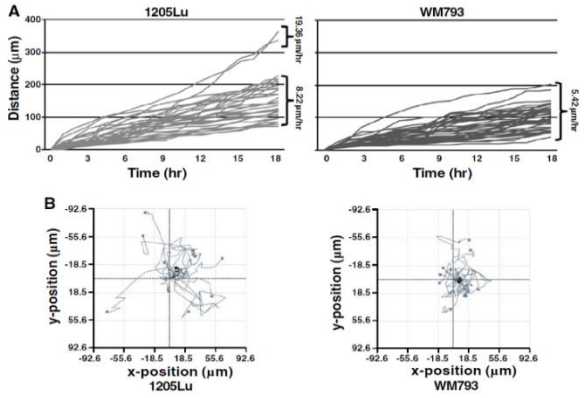


***Figure 4.*** *A) Distance moved for individually tracked cells over time, and B) spatial tracking graphs for the two cell lines.*

The HoloMonitor Wound Healing Assay (fig.5) with automatic calculation of gap width, showed high reproducibility between different image fields on the same gap (fig. 5B) and significant differences between the cell lines using the mean of biological triplicates (fig. 5C). The increasing deviations seen for the 1205Lu cells may be explained by the subpopulation of highly motile cells found in the cell tracking of individual cells presented in fig. 4.

When the motility assays of Hstudio, single cell tracking and wound healing, were compared to the classical Boyden chamber assays, migration and invasion (fig.6), the relative population motility was well comparable between the different assays. The same results were achieved when reanalyzing the DMH data using the new proprietary software App Suite Kinetic Motility assay (blue arrow, fig.6).

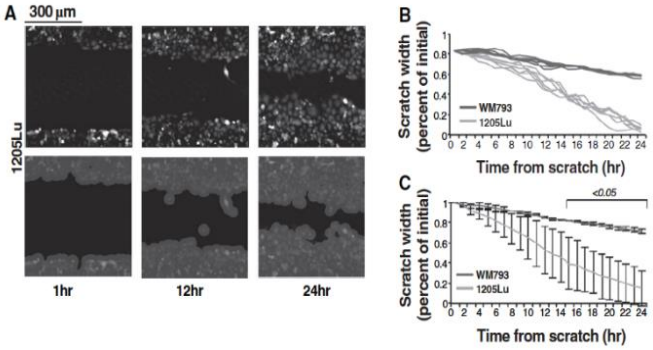


***Figure 5.*** *A) Representative images of wound healing over time, B) Gap width of six replicate image fields from each condition, C) Mean gap width of biological triplicates per condition.*

In our original paper, we also present results from DHC on proliferation and proliferation-associated motility, proving that in the wound healing assay, the gap was filled quicker for the 1205Lu cells than the WM793 cells due to higher motility, not proliferation. This distinction cannot be made using the standard motility and migration methods on populations.

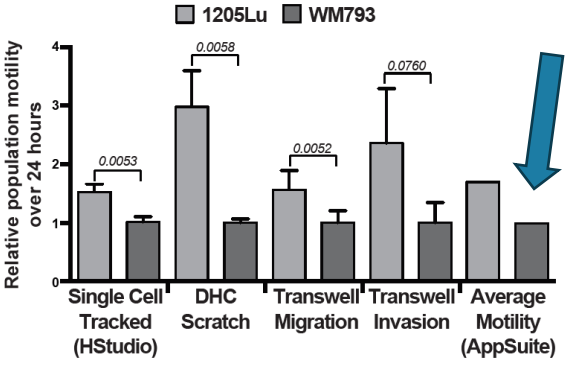


***Figure 6.*** *Relative population motility for 5 assays.*

## CONCLUSIONS

Our results show high correlation between the different evaluated motility assays. There are many benefits of using DHC compared to the standard methods, one being the possibility to capture rare events in sub-populations of cells. The added population-based results obtained from HoloMonitor App Suite Kinetic Motility Assay, also highly correlated to the other population-based results. This enables fast, easy and automatic analysis of population data. The achieved DHC images can later be used in Hstudio to track individual cells regarding movements as well as morphology. Moreover, since DHC is label free, the cells are left unharmed and can be used for further analysis using end-point assays after imaging, to gain even deeper knowledge.

## REFERENCES

1. Mölder et al. (2008), *Non-invasive, label-free cell counting and quantitative analysis of adherent cells using digital holography,* J. Microscopy, 232:240-247, http://dx.doi.org/10.1111/j.1365-2818.2008.02095.x

2. Zhang and Judson (2018) *Evaluation of holographic imaging cytometer HoloMonitor M4 motility applications,* Cytometry, http://dx.doi.org/10.1002/cyto.a.23635