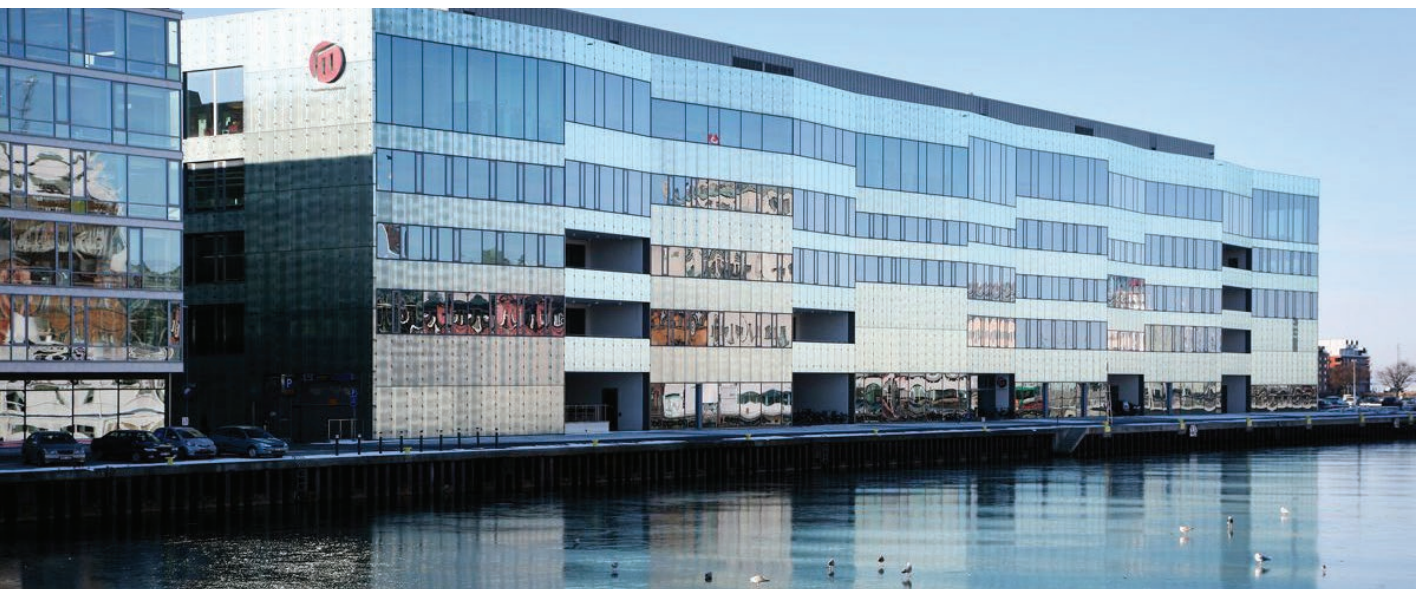


# Application Note

on

## Live cell imaging cytometry of antibody captured suspension cells

Malmö University Series



### FEATURED SCIENTIST

“By using holographic microscopy combined with microarray, each cell morphology parameter was available, not only on population levels, even at the cellular level.”

Zahra El-Schich, Malmö University



**MALMÖ UNIVERSITY**

# Interfacing antibody-based microarrays and holographic microscopy enables label-free detection of cell volume loss

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## ABSTRACT

Suspension cells are challenging for microscopy since they are floating at several different levels in the media. Even more challenging is to analyze these cells in a non-disturbing way. An antibody microarray can capture and immobilize living, unstained suspension cells. Then morphological parameters such as area, optical thickness, volume and many more can be measured using the HoloMonitor<sup>®</sup> imaging cytometer. In this study, after cytotoxic drug treatment of antibody-captured human leukemic T-cells, a time dependent decrease of cell volume, area, thickness, eccentricity and irregularity was observed and quantified.

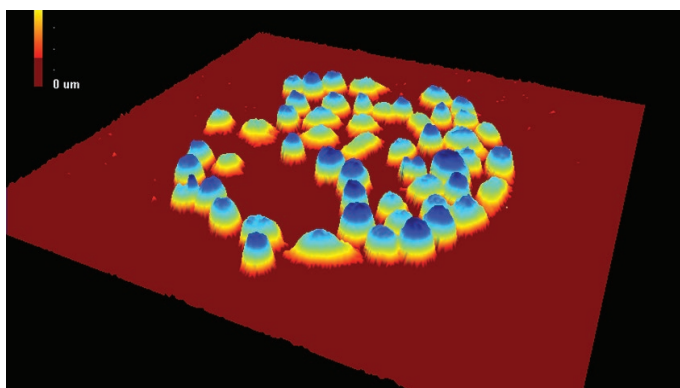
## INTRODUCTION

Blood cancer cells differ from other cancer cell types mainly because they do not co-exist in a tumorous tissue, but are solitary cells or *suspension cells*. Therefore the study of these cells needs other methods than *adherent cells* that adhere to each other and to the bottom of culture vessels.

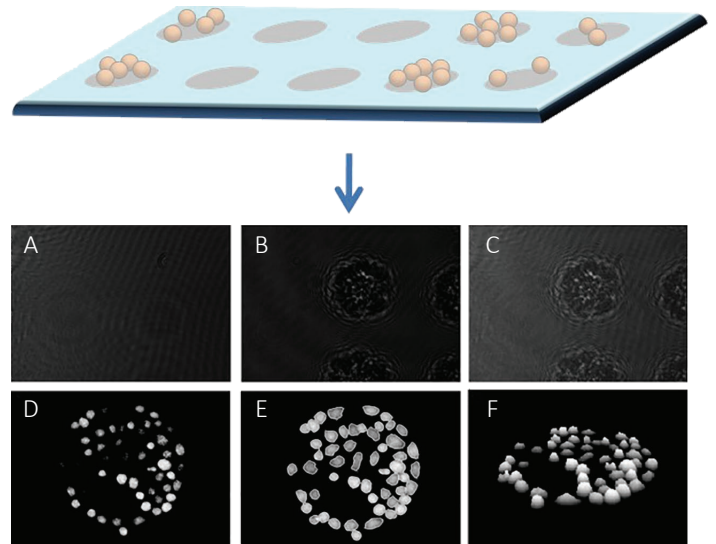
Holographic microscopy (HM) provides a method for label-free morphological analyses of adherent cells directly in their culture environment (Marquet et al 2005). The analyses are based on holographic images where all data are embedded within the image. Therefore all data can be back-traced to its raw-data source. By capturing living suspension cells on antibody-coated microarrays (Wingren et al 2009, Figures 1 and 2), and using HM, these cells can now be analyzed in a non-disturbing way as well.

A similar technique with antibody microarrays and holographic microscopy was used by Stybayeva et al (2010). They used CD4/CD8 antibody specific microarrays to extract and analyze HIV-infected T-cells from blood regarding cell counts and morphology. They found that the ratio of CD4 and CD8 T-cells, an important HIV-diagnostic marker, obtained with holography, was in agreement with conventional methods.

The present study focuses on analyses of morphologic markers for dying and dead leukaemic T-cells and B-cells (results not shown in this document) after treatment with the cytotoxic drug



**Figure 1.** A Holographic 3D representation of leukemic T-cells captured on one spot of the microarray by antibodies.



**Figure 2.** A schematic image of an antibody-coated glass slide with captured suspension cells and holographic images of (A) a raw image of the reference beam, (B) a raw image of the object beam after passing through the cells, (C) the interference pattern created after merging A and B, (D) the calculated holographic image of an antibody-coated spot with captured cells, (E) after computational segmentation where each cell has a border and (F) the 3D representation of D.

etoposide. These markers include changes in area, volume, eccentricity (roundness) and irregularity. In addition, cell counts were performed on treated and untreated cell populations.

## MATERIALS AND METHODS

### Cell line

Lurkat cells (leukemic T-cell) were maintained in complete RPMI1640 medium. Cells (200 000), in 100  $\mu$ l PBS with 0,5% BSA, were applied to an antibody array and incubated at room temperature for 30 min. The array was washed 10 times with 100 $\mu$ l PBS with 0,5% BSA until no cells were found outside the antibody coated spots.

### Antibody microarrays

Antibodies and a negative control were immobilized on silane-coated glass (El-Schich et al 2015). Each slide contained 8 replicas of the antibody+control arrays.

### Cell treatments

The experiments were performed outside the incubator with cover glass to avoid drying and gas exchange. For treated cells, either 30 $\mu$ l of 500 $\mu$ M etoposide in PBS-0,5% BSA or 30 $\mu$ l 10% dimethylsulfoxide (DMSO) was added to the array before covering. For untreated cells, 30 $\mu$ l of PBS with 0,5% BSA was added.

### Live cell imaging

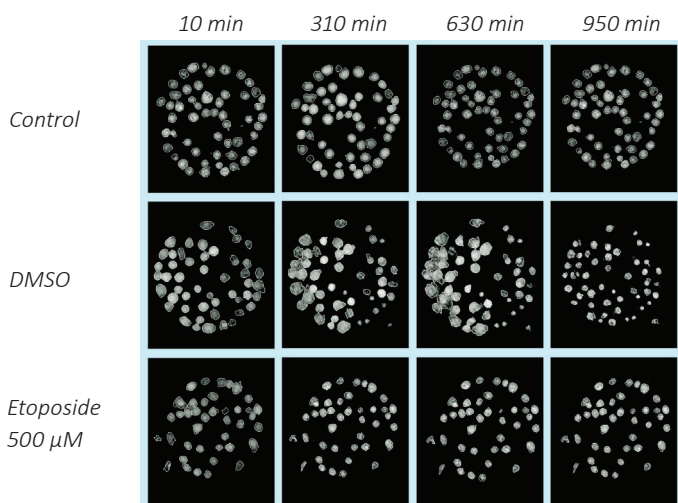
For each experiment, time-lapse sequences with one image captured every 10 minutes for 16h. Each experiment was performed at least twice. The HoloMonitor M2 with a 0,8 mW HeNe laser (633 nm) was used. HoloMonitor Hstudio software was used to analyze the cells regarding different holographic morphologic cell parameters. Optical thickness (optical path length) is based on phase shift, the wavelength of the light and the refractive index of the cell. Optical volume is based on optical thickness and area (Mölder et al).

## RESULTS AND DISCUSSION

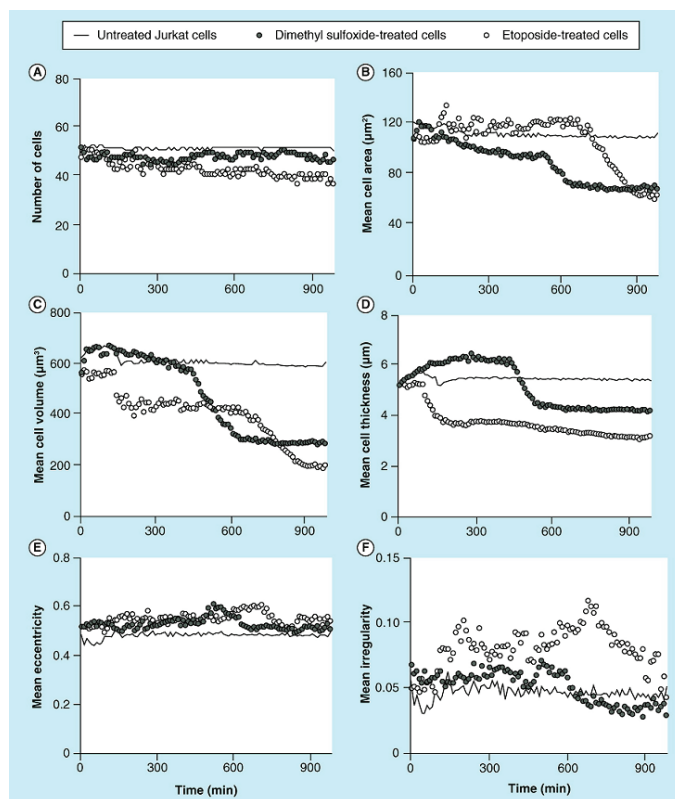
Holographic images of antibody-captured leukemic T-cells (Figure 3) show at a glance the effect of etoposide treatment. The size of etoposide treated- and DMSO-treated cells decreased with time. By using the Hstudio software analysis tool, data on morphology was obtained. As seen in Figure 4, the cell number decreased for etoposide-treated cells. In addition, mean cell area, thickness and volume decreased. The mean eccentricity increased slightly and the mean irregularity increased dramatically. The untreated cells did not change morphologically during the 900 minutes of analysis and the cell counts remained equal at all time points. These findings are in agreement with the hypothesis that the HoloMonitor indeed can measure the changes in morphology of suspension cells that have been induced to die by a cancer drug.

Interestingly DMSO, an often used solvent for drugs in toxicological assays, also affected cell morphology although the cell counts were unaffected. This effect is probably mistakenly overseen in other analysis methods as it is not noticed. Seen in Figure 4, DMSO decreased cell area. Mean volume decreased after approximately 400 minutes of treatment. Mean thickness first increased after approximately 400 minutes and thereafter decreased. Eccentricity and irregularity differ slightly from the untreated cells.

This study shows how live cell imaging can be applied on suspension cells with analysis of the cells regarding morphology while still alive and healthy. Besides capturing the cells at one focus plane for imaging, this method with antibody coated mi-



**Figure 3.** Holographic images of leukemic T-cells on antibody coated microarrays. The images are showing non-treated controls, DMSO- and etoposide-treated cells for 10, 310, 630 and 950 minutes.



**Figure 4.** Morphological cellular parameters obtained with the HoloMonitor of leukemic T-cells over time (minutes). (A) Cell numbers; (B) mean cell area; (C) mean cell volume; (D) mean cell thickness; mean cell eccentricity; and (F) mean cell irregularity.

cro-arrays can be applied to sort out specific cell sub populations carrying specific surface proteins, i.e. CD24, CD8 as studied by Stybayeva et al.

## CONCLUSIONS

Live cell imaging of suspension cells is made possible by specifically capturing them on antibody coated microarrays. We take things even further when using the HoloMonitor image cytometer, as the instrument provides a vast amount of data on each cell in the image. These data can be used for individual cells and/or for whole populations. In addition, all acquired data can be back-traced to each raw data source, a huge advantage in cell analyses, making the obtained results truly trustworthy.

## REFERENCES

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