

Application Note MMI-CS-002 – Live Cell Imaging

Live Cell Imaging on a Whole Slide Scanner

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Abstract

Whole Slide Imaging is becoming increasingly popular in digital pathology as well as in clinical and basic research.

The MMI CellScan system has been developed to combine whole slide imaging with all functionalities of a research microscope.

In this study, we show how the MMI CellScan can be employed for live cell imaging. The ibidi Stage Top Incubation System ensures physiological conditions and thus allows for long term live cell imaging experiments.



Figure 1: MMI CellScan system on the Nikon Ts2R inverted microscope. The system is compatible with many microscope brands and models and can be combined with all MMI systems for various research applications.

Introduction

Whole Slide Imaging is a new technology also known as virtual microscopy. Full microscope slides are scanned by taking images of each field of view across the entire slide. The pictures are then stitched together to generate a single digital image in high resolution.

The whole slide imaging technology rapidly developed as researchers sought to digitally archive tissue slides to avoid color changes or degradation issues due to long-term storage conditions. In addition, telepathology workflows emerged and enabled pathologists to share image files to their colleagues from all over the world in order to discuss non-obvious cases or to consult experts on specific tissues or diseases.

The MMI CellScan is a whole slide imaging system that can utilize all functions of a research microscope. Thus, scanning is not limited to one resolution, but can utilize objectives optimized for different samples. Moreover, the system can easily switch between brightfield and fluorescence imaging modes with just one mouse click. The MMI CellScan, in addition to standard microscopy slides, is compatible with various sample formats, such as MMI Membrane Slides for laser microdissection as well as well slides, plates, and dishes, making this a highly

versatile and flexible high resolution scanning system.

Here, we demonstrate how the MMI CellScan can also be employed for live cell imaging. The MMI CellScan system can be combined with the ibidi Stage Top Incubation System to ensure that cells remain in physiological conditions during the entire process.

Material and Methods

HeLa cells were cultivated in Dulbecco's Modified Eagle's medium (DMEM), supplemented with FBS, non-essential amino acids, L-Glutamine and Penicillin-Streptomycin at 37 °C and 10 % CO₂. Each 400 µl of a cell suspension with 1 x 10⁴ cells/ml were transferred into the reservoirs of a µ-Slide 8 Well (Cat No 80826 at ibidi; Product No 50114 at MMI) and grown for 24 h.

For whole slide imaging, the µ-Slide 8 Well was transferred to the MMI CellScan equipped with the ibidi Stage Top Incubation System which was equilibrated to 37 °C, 10 % CO₂ and 70 % humidity. The slide was then

scanned at a magnification of 20 x. With the time lapse function implemented in MMI CellScan, images were automatically scanned for 24 hours with one image per 30 min. During scanning, the single field of view pictures were seamlessly stitched to form one full resolution image per time point. All images were then analyzed using the MMI CellViewer software.

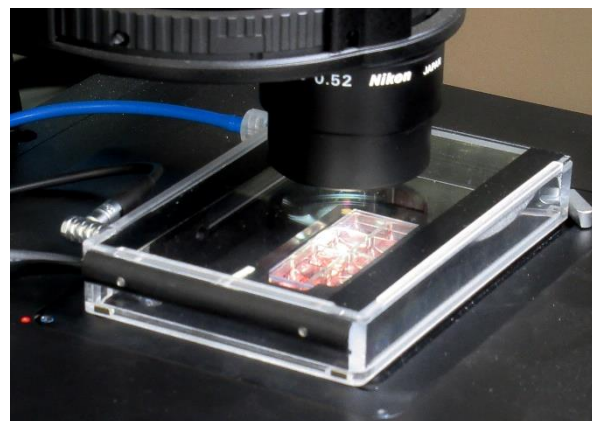


Figure 2: Experimental set up used in this study: The ibidi Stage Top Incubation System was mounted on the MMI CellScan. The transparent incubation chamber encloses the µ-Slide 8 Well with the HeLa cells.

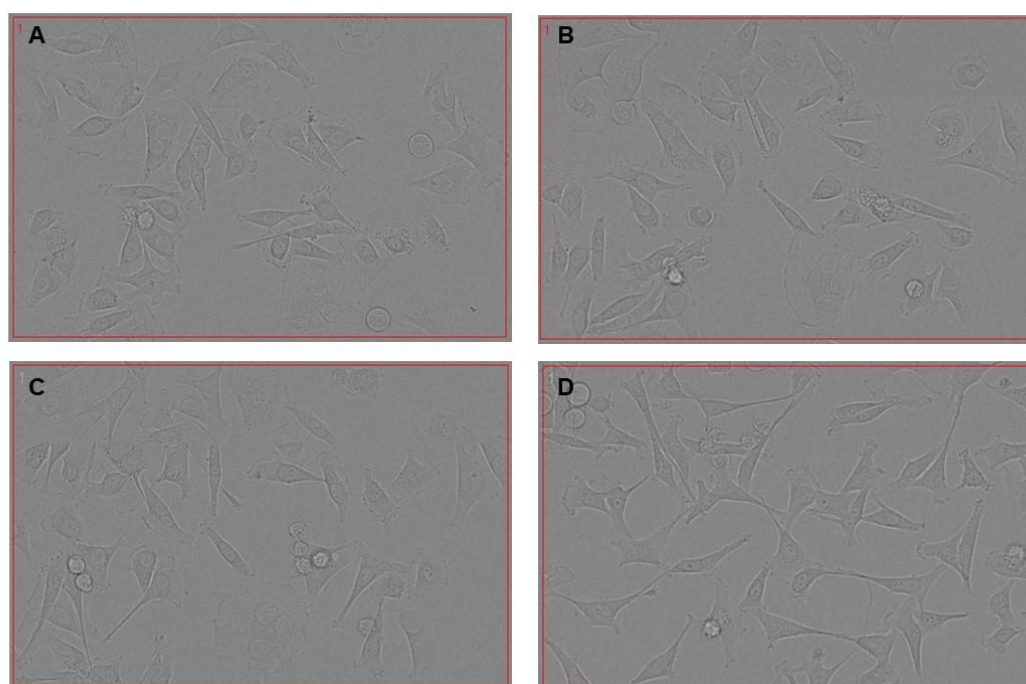


Figure 3: HeLa cells monitored over several hours using the MMI CellScan. A) Image at day 1, 4 pm. B) Image at day 1, 5.30 pm. C) Image at day 1, 7.30 pm. D) Image at day 2, 4 pm.

Results

The MMI CellScan is a microscope-based slide scanner which can readily be used for various applications. Here, we show that the MMI CellScan can also be employed for live cell and time lapse imaging applications.

The MMI CellTools software offers different features and parameter settings to efficiently scan one or several slides automatically providing optimal image quality. To enable live cell imaging application, a time lapse option has been integrated to take images at predefined time points.

To ensure fully physiological conditions throughout the live cell imaging process, the MMI CellScan is combined with the ibidi Stage Top Incubation System. This system is able to continuously adjust the concentration of CO₂ as well as temperature and humidity values to provide cells with optimal growth conditions throughout the experiment.

To test the capabilities and performance of the MMI CellScan live cell imaging set up, HeLa cells were grown in μ -Slide 8 Well and subjected to time lapse whole slide imaging over a time span of 24 hours. Images were taken every 30 min and subsequently analyzed using the free MMI CellViewer software.

As the MMI CellScan saves images with absolute position information, individual cells can easily be monitored. We found that the cells were viable throughout the experiment. In addition, we could observe that the cells changed their morphology and they are migrating within the cell culture well.

Discussion

In this study, we demonstrate that the MMI CellScan system is able to perform live cell imaging experiments in fully physiological cell culture conditions. The cells were viable after the experiment and can thus be further cultured. Alternatively, the cells can be subjected to single cell isolation procedures. The MMI CellEctor, for example, is able to pick single adherent cells from μ -Slide 8 Well *. The cells can then be further cultivated or subjected to transcriptomics or proteomics analysis as well as to sequencing technologies.

With the novel live cell imaging functionality, the MMI CellScan expands its application range beyond whole slide imaging to now include a vast variety of cell biology research applications. At the same time, the MMI CellScan can also be employed as a standard (fluorescence) research microscope, thus combining microscopy, live cell imaging and slide scanning in one single instrument.

* Please find further information on the MMI CellEctor on our website or contact us for further information.

www.molecular-machines.com/products/cellector
info@molecular-machines.com

MMI - your partner providing unique competence in microdissection – micromanipulation - imaging. We offer

- Capillary-based selective isolation of single cells from suspension (CellEctor)
- PicoCut laser microdissection to isolate cells in tissue (CellCut)
- Microscopy-integrated Whole Slide Imaging (CellScan)
- Optical tweezers to quantify biological forces (CellManipulator)

Contact us via: info@molecular-machines.com