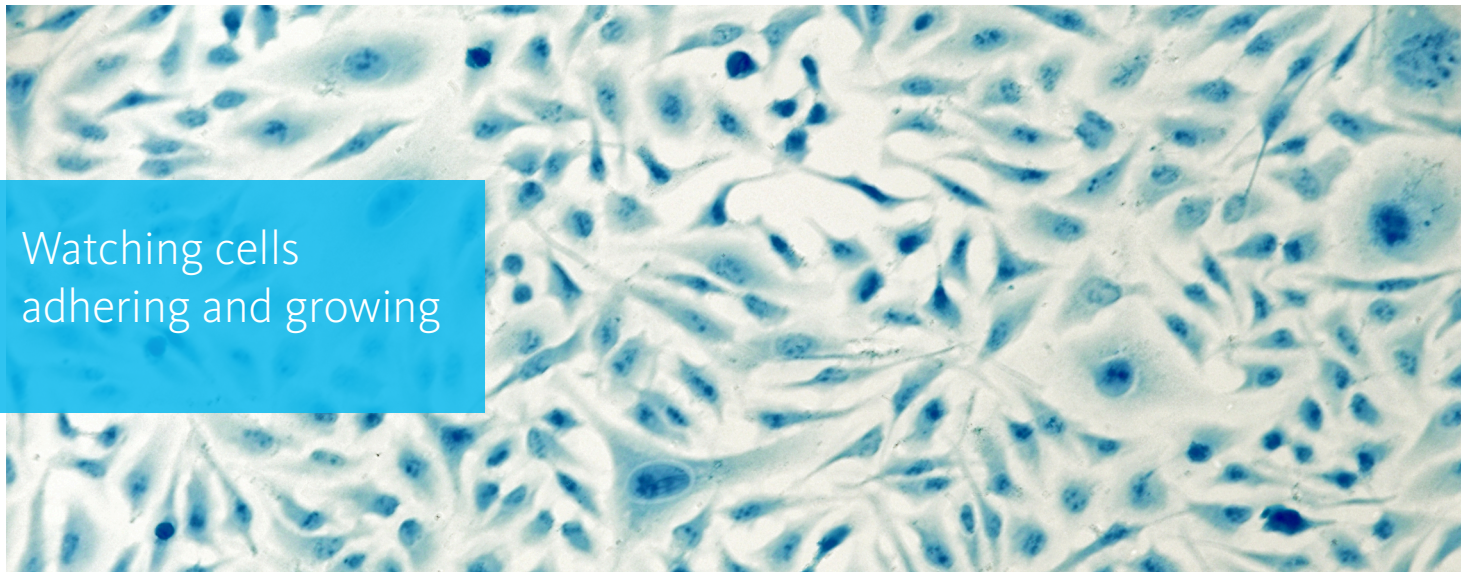
Application Note

Long-term Live Cell Imaging on a Whole Slide Scanning Microscope



Watching cells adhering and growing

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Abstract

Monitoring cells over several days under physiological conditions is essential for many cell-based assays and culture studies. Stage Top Incubators are frequently utilized on microscope stages to maintain critical parameters such as temperature, CO_2 concentration, and humidity consistently over time. This setup allows the study of cells over long periods of time under physiological conditions directly on the microscope. In this study, we take the next step to combine live cell studies with Whole Slide Imaging (WSI). The entire petri dish is scanned in full resolution at pre-defined time points. Thus, all cells can be tracked seamlessly and no information is lost.



Fig. 1: MMI CellScan system on the Nikon Ts2R inverted microscope equipped with the Okolab Stage Top Incubator. Both the MMI CellScan as well as the Okolab Stage Top Incubator are compatible with many microscope brands and models, and can be combined with all MMI systems for various research applications.

Introduction

Continuous observation of living cells is a prerequisite for many cell-based assays and cell culture studies. Stage Top Incubators maintain cells under physiological conditions with constant temperature, humidity, and CO_2 concentration, enabling observation on a microscope over extended periods.

Whole Slide Imaging is a technology wherein full microscope slides are scanned by taking images of each field of view across the the entire slide. The pictures are then stitched together to generate a single digital image in high resolution.

The MMI CellScan is a whole slide imaging system that can utilize all functions of a research grade microscope. Therefore, in

addition to being able to digitally archive whole slides, it can also capture large areas from a various sample formats such as MMI membrane slides for laser microdissection, well slides, plates, and dishes to accommodate living cells. Furthermore, scanning is not limited to a single resolution because the MMI CellScan can utilize any objective and optimize the aquisition for the sample of interest. Moreover, the system can easily switch between brightfield and fluorescence modes with just one mouse click, making it a highly versatile, intuitive, and flexible high-resolution scanning system.

In this study, we demonstrate how the MMI CellScan can be employed for long-term imaging of living cells. The MMI CellScan system was used with the Nikon Ts2R microscope, which was equipped with the Okolab Stage Top Incubator to ensure that cells remained under physiological conditions throughout the entire process (Figure 1).

humidity. Temperature, CO_2 and humidity controllers were configured using the Oko Touch touch screen interface. The µ Dish 35 mm was then scanned at a magnification of 20 X. With the time lapse function implemented in MMI CellScan, images were automatically scanned for 72 hours with one image per 60 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 free MMI CellViewer software which is optimized for large datasets.

Results

The MMI CellScan is a microscope-based slide scanner that can be used for a range of applications. Here, we demonstrate that, in addition to whole slide imaging, the MMI CellScan can also be employed for live cell and time-lapse imaging applications.

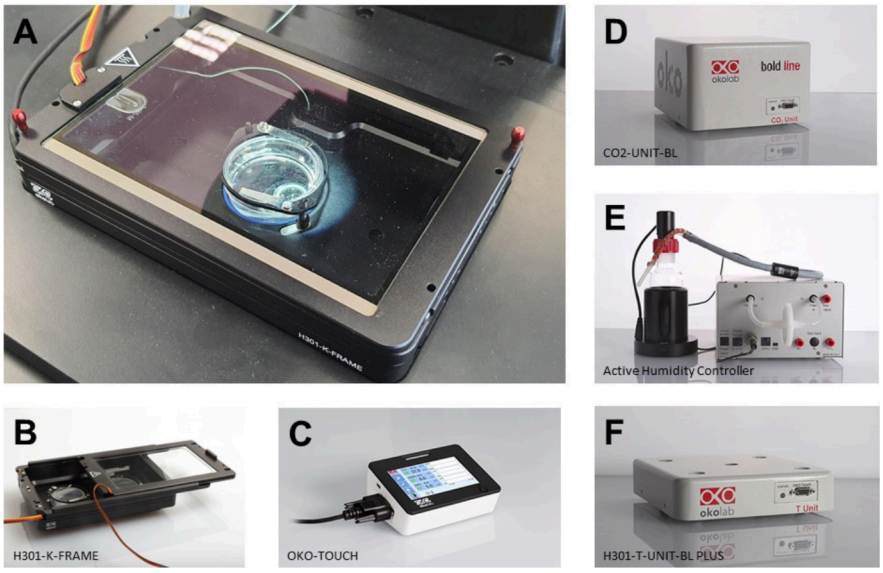


Fig. 2: Experimental setup used in this study: **A)** The Okolab Stage Top Incubator was mounted onto the Märzhäuser stage on the MMI CellScan. The transparent incubation chamber encloses the µ-Dish 35 mm with HeLa cells. The Okolab incubator contains following components: **B)** H301-K-Frame mounted on microscopestage to hold the sample and to establish controlled conditions, **C)** Oko-Touch touch pad to set and control all parameters during the experiment, **D)** CO_2 –Unit-BL to regulate CO_2 concentration in the K-Frame, **E)** ActiveHumidity Controller to control humidity inside the K-Frame and **F)** H301-T-Unit-BL Plus to adjust the temperature within the K-Frame.

Material and Methods

A FFP HeLa cells were split and transferred into Dulbecco's Modified Eagle's medium(DMEM), supplemented with 10% FBS, supplemented with non-essential amino acids, L-Glutamine, and Penicillin-Streptomycin. 2 ml of a cell suspension with 1 X 104 cells/mlwere transferred into a µ-Dish 335 mm (Cat No81156 at ibidi). 1 µg/ml propidium iodide were added as a cell viability marker. For whole slide imaging, the µ-Dish 35 mm was transferred to the MMI CellScan Ts2R microscope equipped with the Okolab StageTop Incubator (Figure 2) which was equilibrated to 37°C, 10 % CO_2 and 90%

The MMI CellTools software offers a range of features, including 5D imaging (XY-scan, Z-stacks, time-lapse, and multi-fluorescence), focus mapping, multiple regions of interest, and parameter settings to efficiently scan one or several slides automatically, providing optimal image quality. To enable live cell imaging, a time-lapse option has been integrated to capture images at predefined time points. To ensure fully physiological conditions throughout the live cell imaging process, the MMI CellScan can be combined with the Okolab Stage Top Incubator (Figure 2). This system continuously adjusts the concentration of CO_2, as well as temperature and humidity levels, to provide cells with optimal growth conditions throughout the experiment.

To test the capabilities and performance of the MMI CellScan live cell imaging setup, HeLa cells were grown in a µ-Dish 35 mm and subjected to time-lapse whole slide imaging over a period of 72 hours. Images were taken every 60 minutes and subsequently analyzed using the free MMI CellViewer software. The MMI CellScan saves images with absolute position information, allowing individual cells to be easily monitored. The MMI CellViewer loads the first image of the time-lapse series in an overview mode, where the image can be zoomed and moved using intuitive software features. With the time-control slider, images from different time points are loaded to display the same field of view at the same magnification.

We found that the HeLa cells remained viable throughout the experiment. Additionally, we observed that the cells settled, changed their morphology, and began to grow and divide in the cell culture dish (Figure 3). Only a small fraction of the cells were not viable and retained their round shape after 3 days of observation. Dead cells were stained orange due to the propidium iodide in the medium, while viable cells continued to grow and divide throughout the entire experimental period.

Discussion

In the present study, we demonstrate that the MMI CellScan system can be equipped with the Okolab Stage Top Incubator to enable long-term live cell imaging experiments under fully physiological cell culture conditions with constant temperature, humidity, and CO_2 concentration. As the cells remained viable and continued dividing throughout the entire experiment, the MMI CellScan/Okolab incubator platform can provide meaningful insights into cell cycle experiments, cell migration, co-culturing, and other cell-based assays. This instrument surpasses currently available whole slide imaging systems, as its time-lapse functionality is not limited to a single field of view but encompasses the entire sample vessel, such as petri dishes or multi-well plates, thus providing a comprehensive spatio-temporal data set of the full sample. Scanning of living cells is not confined to brightfield illumination; it can also be performed using multi-color fluorescence, which is particularly valuable when working with multiple cell types in the assay. With its novel live cell imaging capabilities, the MMI CellScan expands its application range beyond whole slide imaging to include a vast variety of cell biology research applications.

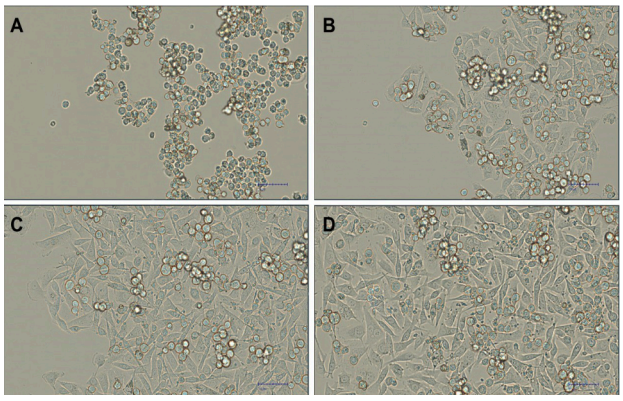


Fig. 3: HeLa cells monitored over 72 hours using the MMI CellScan. **A)** Image at day 1, 10 p.m. **B)** Image at day 2, 5 p.m. **C)** Image at day 3, 2 am. **D)** Image at day 3, 5 p.m.

Your benefits

- Time lapse function captures dynamic changes in cells over extended periods, providing a detailed view of cellular processes such as migration, division, and morphology changes
- CellScan is the whole slide scanner with the highest flexibility: Compatible with all objectives, numerous research microscopes and all possible imaging modes
- High-resolution imaging of the entire sample, including complex structures and interactions across multiple fields of view
- High-performance whole slide scanner:Less than 60 seconds to scan a 15 x 15 mm section (20x objective)

A quick motion video including all time frames can be viewed here. The video displays the same field of view as depicted in Figure 3.

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