Application Note MMI-CS-006 – Live Cell Imaging

Long-term Live Cell Imaging on a Whole Slide Scanning Microscope: Watching cells adhering and growing

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Abstract

Being able to monitor cells over several days under physiological conditions is a prerequisite for many cell-based assays and cell culture studies.

Stage Top Incubators are commonly used on microscope stages to keep important parameters constant over time, such as temperature, CO_2 concentration and humidity. 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.

Introduction

Continuous observation of living cells is a prerequisite for many cell-based assays and cell culture studies.

Figure 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.

Stage Top Incubators maintain cells under physiological conditions with constant temperature, humidity and CO_2 concentration,

and enable inspection on a microscope over extended periods.

Whole Slide Imaging is a new technology wherein 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 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 variety of 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 one 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 this 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 has been equipped with the Okolab Stage Top Incubator to ensure that cells remain under physiological conditions during the entire process (Figure 1).

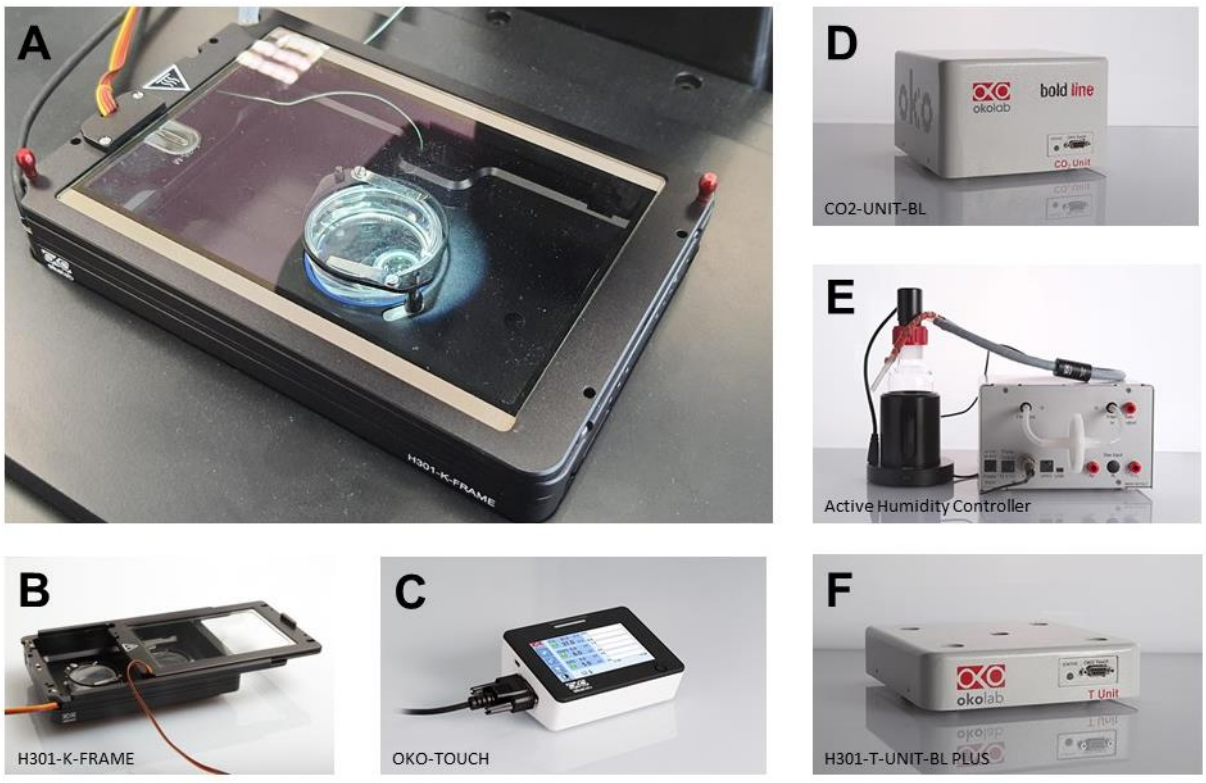

Figure 2: Experimental set up used in this study: A) The Okolab Stage Top Incubator was mounted onto the Märzhäuser stage on the MMI CellScan. The transparent incubation chamber encloses the µ-Dish 35 mm with HeLa cells. The Okolab incubator contains following components: B) H301-K-Frame mounted on microscope stage 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) Active Humidity 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

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 10^4 cells/ml were transferred into a µ-Dish 35 mm (Cat No 81156 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 Stage Top Incubator (Figure 2) which was equilibrated to 37 °C, 10 % CO_2 and 90 % 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 which can be used for a range of applications. Here, we show that in addition to whole slide imaging, the MMI CellScan can also be employed for live cell and time lapse imaging applications.

The MMI CellTools software offers a range of features, including, 5D (XYZtλ), focus map, multiple regions of interest, etc., 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 take 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 is able to continuously adjust the concentration of CO_2 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-Dish 35 mm and subjected to time lapse whole slide imaging over a time span of 72 hours. Images were taken every 60 min and subsequently analyzed using the free MMI CellViewer software.

The MMI CellScan saves images with absolute position information. Therefore, individual cells can easily be monitored. The MMI CellViewer loads the first image of the time lapse series in an overview mode. The image can be zoomed and moved by intuitive software features. Using the time-control slider, images of the time points are loaded to display the same field of view with the same magnification.

We found that the HeLa cells were viable throughout the experiment. In addition, we could observe that the cells settled down, changed their morphology and started to grow and divide in the cell culture dish (Figure 3). Only a small fraction of the cells were not viable and stayed in their round shape after 3 days of observation. Dead cells are stained orange due to the propidium iodide in the medium. However, 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 in fully physiological cell culture conditions with constant temperature, humidity and CO_2

concentration. As the cells were viable and dividing throughout the entire experiment, experiments performed on the MMI CellScan/ Okolab incubator platform can provide meaningful insights into live cycle experiments, cell migration, co-culturing or other cell-based assays.

The instrument exceeds currently available whole slide imaging systems as the time lapse functionality is not limited to a single field of view, but includes 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 living cells is not limited to brightfield illumination but can also be performed as multi-color fluorescence which provides valuable information especially when working with more than one cell type in the assay. 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.

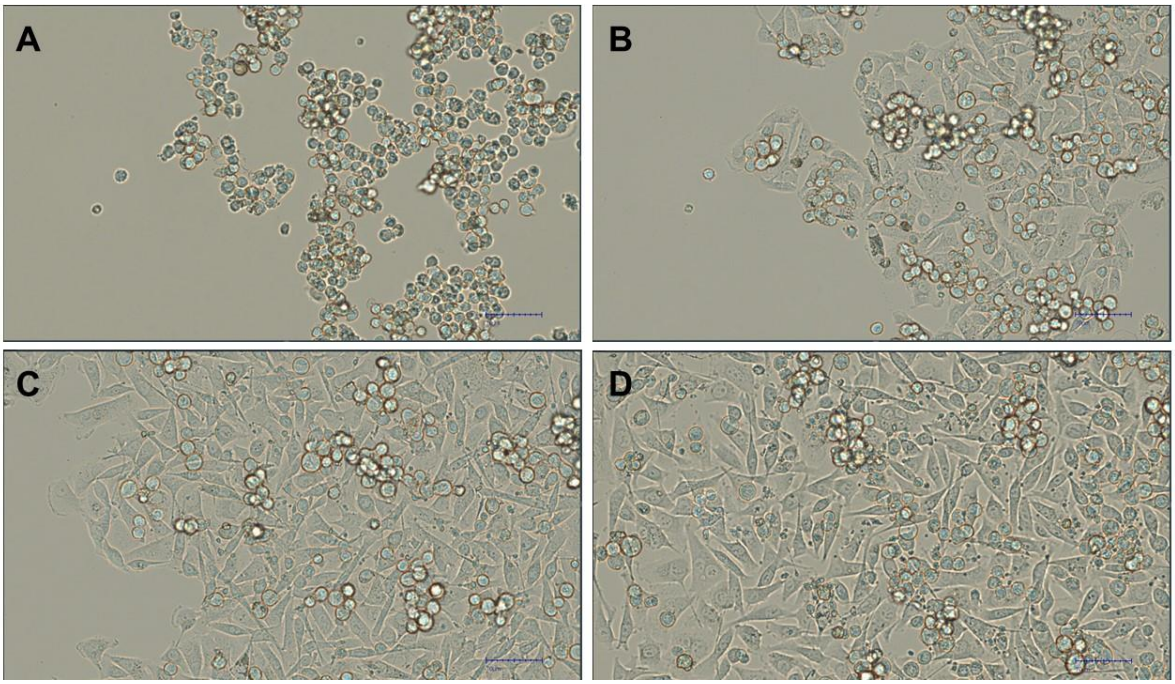

Figure 3: HeLa cells monitored over 72 hours using the MMI CellScan. A) Image at day 1, 10 pm. B) Image at day 2, 5 pm. C) Image at day 3, 2 am. D) Image at day 3, 5 pm.

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.

https://www.youtube.com/watch?v=fNll32qzhsA