Lensless Microscopy of Cell Cultures

Holographic Live Cell Imaging in New Dimensions

The lensless Cell-Microscope combines holographic imaging of cells with a thermoelectric cooling system and automated cell segmentation for live cell imaging inside the incubator. Using holography instead of optical focusing enables to build a cost-efficient, robust and compact microscope. The cooling of the CMOS camera defines the local temperature of the integrated cell culture chamber. Thousands of cells can be imaged, segmented, counted and tracked continuously within the large field of view.

With the cultivation of an immortal cell line in vitro, back in the 1950's, a complete new field of sciences arose, whose most extensive use is still to come. Cell cultures allow studying the behavior of cells under different influences and under defined boundary conditions, starting from a single cell to complex tissues. An enormous increase in cell-based assays can be expected in the future, due to further developments in the fields of regenerative medicine, tissue engineering and cell therapy.

Cells under Observation

Live cell imaging with a cell-microscope is the most important analysis tool to study cells. New approaches in optical microscopy have pushed the resolution far beyond the diffraction limit. Nevertheless with lens-based microscopes demand a compromise between resolution and field of view. A low magnification offers statistically significant cell numbers but at the expense of a lower resolution. This article presents a new method for live cell imaging for meaningful cell cultures assays without the need for additional laboratory equipment, such as microscope incubation chambers [1]. With lensless microscopy, it is possible to image consecutive cells over a wide field of view by use of holography [2][3]. The digitalized cell holograms provide a higher information density than conventional light microscope images. Besides high contrast imaging, without the need of label or phase contrast, it is possible to determine the object distance and distinguish the adhesion status of the cells (fig.2). This is an important criterion to evaluate the biocompatibility of adherent cell cultures and is not visible with conventional phase contrast microscopes [4].
Furthermore the holograms can be digitally reconstructed using a double inverse Fourier transform into focused images with variable focal distance, due to the inherent three dimensional image information [5] (fig. 3).

**Lensless Cell-Microscopy**

The lensless microscopy is based on in-line holography. A red laser diode emits coherent light, which represents the reference wave. At a distance of ~4 cm the wave front is scattered at cells inside the culture channel, resulting in the object wave. The unscattered reference wave interferes at the 750 μm underlying sensor surface with the object wave. A 10 MP CMOS sensor digitalizes the individual cell holograms [6]. Because the system is not limited by lenses, the resulting field-of-view equates to the sensor dimensions of 29.4 mm², which is an order of magnitude higher than comparable light microscopes with 4x objective (fig. 2 left). The lensless Cell-Microscope fits easily in an incubator due to its minimal size of 12 cm x 6 cm x 10 cm (fig. 4 middle).

**Thermoelectric Cooling of the Microscope Camera**

In order to avoid thermal effects on the cells, the camera temperature is kept at 37°C by Peltier cooling. The thermoelectric cooler is mounted underneath the board camera. A temperature sensor is attached to the image sensor and measures continuously the temperature. The temperature data are logged and evaluated by an external temperature controller, which adjusts the cooling power if required. Consecutive image capture with a self-programmed acquisition software enables time-lapse recording of the cell cultures over course of several days. The image capture causes additional heat in the camera, primarily in the CMOS sensor. In order to avoid a rise in temperature in the culture chamber, the camera software sends a signal to the temperature controller to initiate precooling of the board camera shortly before each image acquisition. Temperature measurement inside
the cell channel showed only a small temperature offset of 0.3°C, which lies within the temperature tolerance of the used incubator and can therefore be neglected.

**Lensless Live Cell Imaging of Cell Cultures**

The *in vitro* cell growth of a fibroblast L-929 culture was recorded every 10 minutes over 7 days in more than 1000 pictures and visualized as a time-lapse video (fig. 1 - see video). The captured images show apart from the cell location and dimension also the adhesion status. While adherent cells have a bright internal spot, nonadherent cells have a globular shape with a different refraction resulting in a darker core area. This is an useful feature for the detection of dividing or dead cells and to determine the division rate of the observed cell culture [2] (fig. 2 right). Automated segmentation of the different cell holograms with the open-source image processing program LineageTracker enables to count cells and to plot a growth curve, which illustrates the cell proliferation (fig. 4 right). The verification of the segmentation algorithm for adherent cells showed a high sensitivity of 95% and specificity of 98% (fig. 5 left). The plotted cell growth of a fibroblast culture reveals no influence of the Cell-Microscope with uninhibited growth in the exponential phase. The holographic Cell-Microscopy can therefore be used for automated cytotoxicity tests with real-time analysis of the cell proliferation kinetics. The relevance of this method is emphasized by the ability to monitor up to 20,000 cells of a confluent cell layer within just one image. We also demonstrated that it is possible to capture the whole cell culture channel of 2.5 cm² by image stitching and to count cell numbers, which was only feasible by flow cytometry up until now [1].

**Cell Tracking**

The segmented cells can be tracked over the whole experiment time to visualize cell migration, velocity, division rate and the cell lineage [7]. These characteristics are used in chemotaxis assays, for example to analyze cell migration and division in wound healing assays [3]. We visualized the tracks of several cells and noticed that, for instance mother cells, which cover a long distance between cell divisions hand down this attribute to their daughter cells (fig. 5 middle - see video).

**Temperature Induced Growth Inhibition**

The small distance of 750 μm between culture chamber and image sensor leads to a nearly direct thermal contact. That is why the local temperature of the cell culture chamber can be controlled by the Peltier cooling system in a range from about 10°C - 60°C. We showed that increasing the temperature of the CMOS sensor to 39.5°C for 20 s reduced the local cell growth for 4 hours and afterwards it returns back to exponential growth (fig. 4 right). During that period no dead cells were
**Mutated Cells with Abnormal Cell Division**

With the large cell number inside the field-of-view it is also more likely to observe rare events in the cell culture, such as tripolar cell division of mutated cells. The unusual division was easy to identify in the hologram and was reconstructed for detailed examination (fig. 5 right - see video). Analyzing the degenerated cell in the time-lapse video, it became clear, that the dividing cells could not separate during the cytokinesis and merge in one cell body again. Consecutive cell divisions showed identical malfunctions. The uncompleted cell division leads to a giant cell with several cell nuclei and with 20-times the size of an average adherent fibroblast.

**Conclusion**
The lensless Cell-Microscope offers great potential for automatically monitoring cell cultures, analyzing their behavior and to quantify the proliferation dynamics for cell assays, cytotoxicity tests and drug testing in a compact and cost-efficient microscope system.

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**References**

**See videos:**
- video to figure 1
- video to figure 5, middle
- video to figure 5, right

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