# Application Note: Cell Growth Analysis

## Cell Growth Analysis using the 24-channel microscope zenCELL owl

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### Introduction

In order to conduct reproducible, significant and objective experiments it is essential to ensure consistent cell culture quality. The base for standardizable analysis is formed by defined and stable environmental conditions including temperature, $CO_2$-supply and the humidity inside the cell incubator.

However, an experiment's reproducibility and validity does not only depend on defined exterior growth conditions but also on the objective assessment whether a cell culture has reached a suitable confluence.

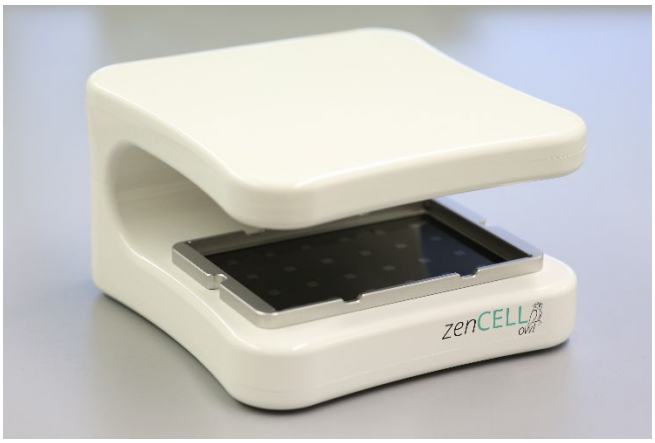



**Figure 1: zenCELL owl.** 24-channel microscope with magnetic adapter frame for 24-well cell culture plate.

Until recently, the decision whether the cell confluence is sufficient for experiments has been determined by users themselves and was thus dependent on the user's estimate. Furthermore, a considerable amount of time has to be spent for manual quality control of cell cultures. Finally, the influence that regular removal of cell cultures from their standardized surroundings inside the incubator might have on cell culture quality has not yet been calculated.

The new 24-channel microscope zenCELL owl (Figure 1) provides the opportunity to circumvent any factors that could influence cell culture quality and thus subsequent experiments.

The zenCELL owl is a 24-channel microscope designed for fast and automated cell culture microscopy. Combining stability and small size it is perfectly suitable for use in incubators. The modular design allows flexible configurations to ensure a secure analysis of biological samples. The small size leaves enough space in the incubator for other cell cultures or more zenCell owl systems.

Integrated image processing algorithms allow for a continually long-term observations and provide fast and concise results concerning the quality of the analyzed cell culture.

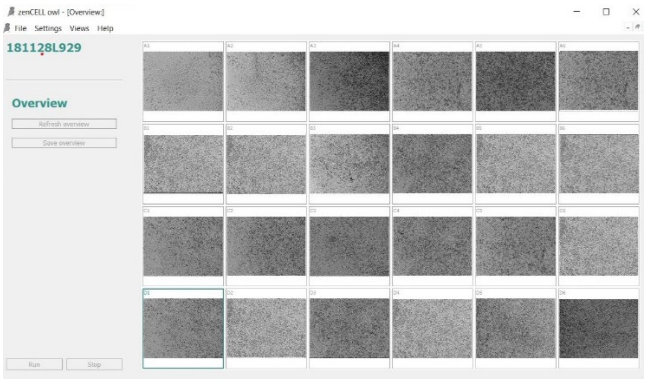



**Figure 2: Footage of cell culture quality.** Parallel imaging is created of each of the 24 wells at fixed intervals.

### Generation of data

A software especially developed for the zenCELL owl (Figure 1) determines the current cell count and the cell coverage of the substrate's surface of the section enlarged by the microscope (1,2 mm x 0,9 mm) via a real-time data analysis. The output includes the number of cells attached to the substrate and the cells detached from it. Simultaneously the microscope documents the quality of each of these individual cell cultures using image recordings (Figure 3). Analysis happens in all 24 wells of a standard cell culture plate at the same time (Figure 2). The recording interval can be set from 10 min to several hours max.

The increase of cell count generally corresponds to the cell coverage of the substrate's surface. Based on that data conclusions concerning the confluence of the cell culture at each point of the analysis can be drawn (Figure 4).

Simultaneous analysis of 24 cell cultures enables users to examine different test conditions at the same time and to compare them directly. This allows for a statistical evaluation of research data (Figure 5).

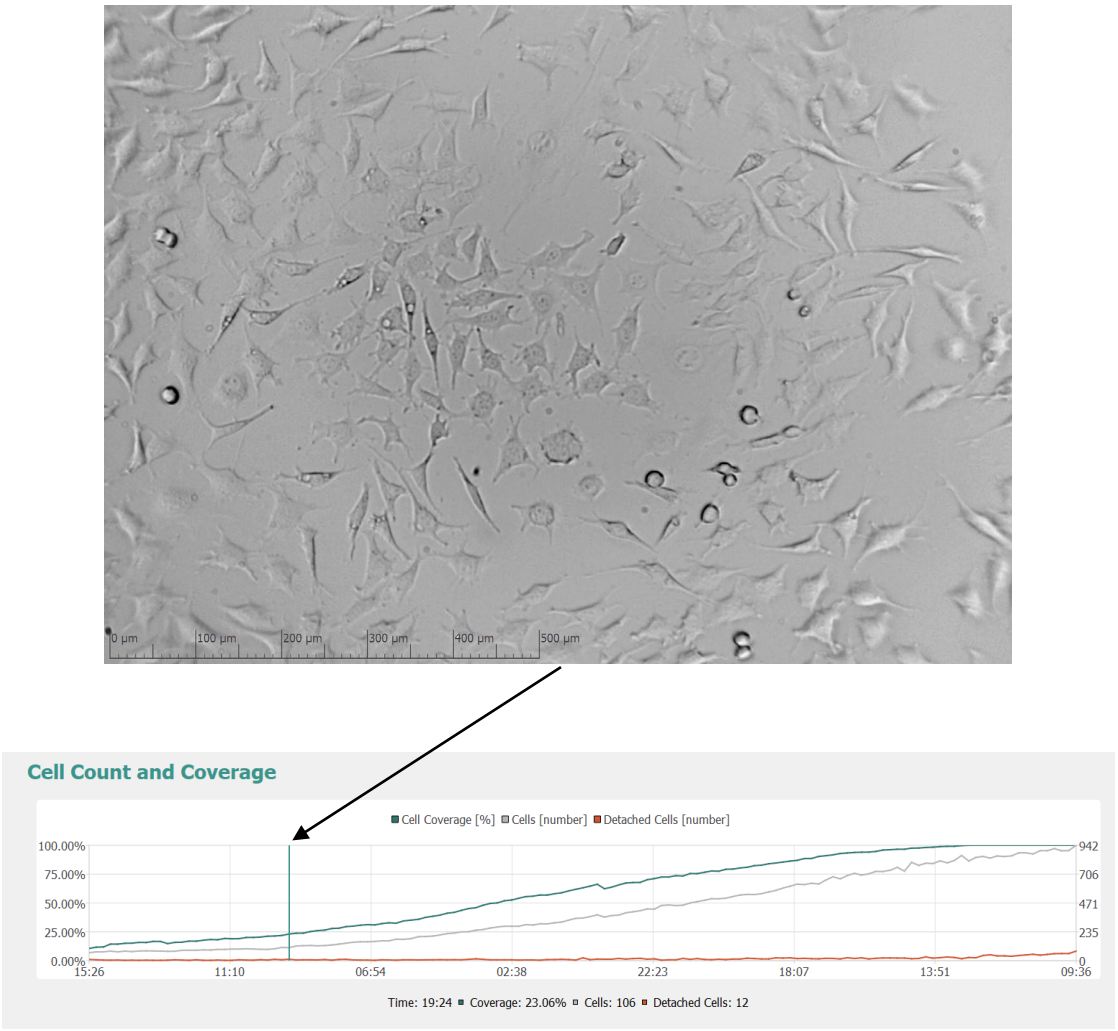



**Figure 3: Single-well-analysis.** Image recording choice intervals (above) in contrast to cell count and coverage (below)

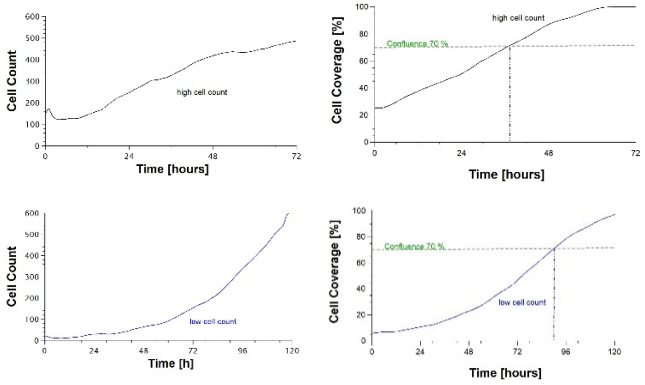



**Figure 4: Correlation of cell count and confluence.** Correlation of cell count and confluence exemplified by two cell cultures with different cell numbers.

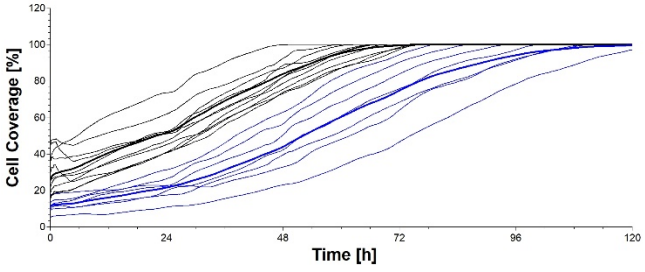



**Figure 5: Growth vs. Time.** Cell coverage of cultures with different cell count. Black: high cell count, blue: low cell count. Thick lines represent mean value of each condition.

### Analysis of cell growth and cell confluence

Under optimal conditions cell lines show their typical growth properties, here shown by an L929 mouse fibroblast cell line. After seeding, cells are grown for 24 hours in the incubator to allow attachment to the substrate. The further growth is observed by the 24 channel microscope zenCELL owl over a period of 120 hours.

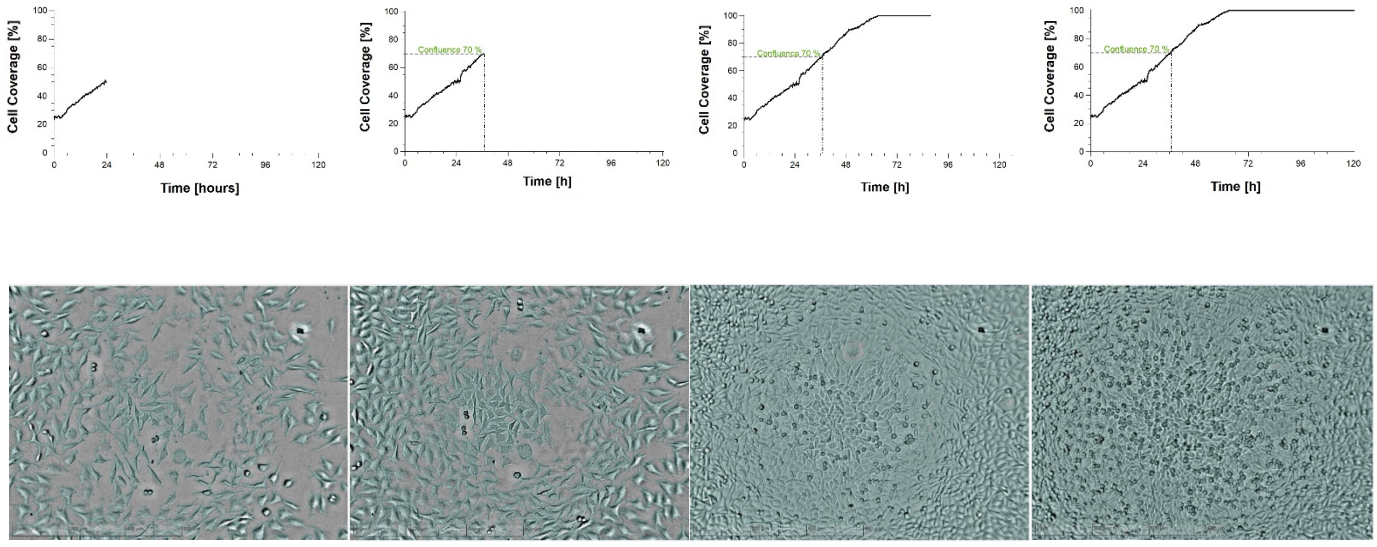
At the start of the measurement cells show the typical proliferation phase which is characterized by constant cell growth (Figure 6a). The higher cell count shows a linear increase of the coverage of the substrate. After about 37 hours there will be a confluence of 70% (Figure 6b) while full coverage is reached after 60 hours (Figure 6c).

Respectively, the initially lower cell count, by comparison, shows a slower increase in coverage and cell count. A confluence of 70% is reached after 90 hours (Figure 6c) while complete coverage of the substrate is achieved after about 120 hours (Figure 6d).

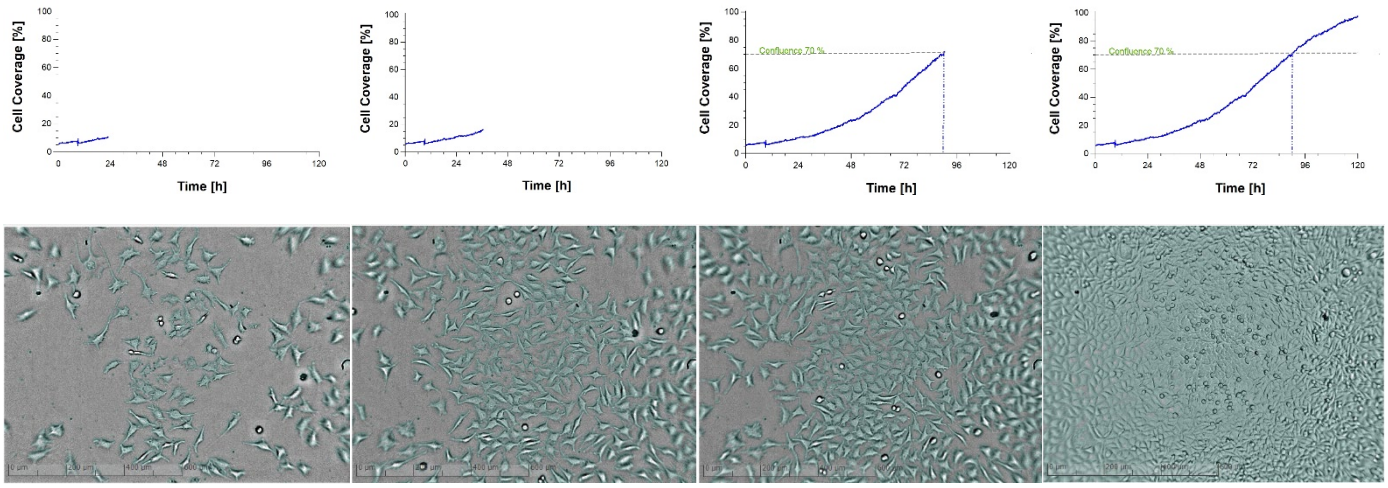
After arriving at a confluent monolayer the cells' proliferation activity decreases due to contact-inhibition. In the plateau phase that follows cell division and cell death are in balance which results in a stagnant cell count.

As the illustrations show, the zenCELL owl is capable of exactly determining the exact moment at which a specified confluence of the individual wells is reached (Figure 6, Figure 7).

**High cell count**:




**Low cell count:**




**a** **b** **c** **d**

**Figure 6: Growth of cultures with different cell counts.** a) proliferation phase of both cell cultures. b) culture with higher cell count reaches confluence of 70 % (above). Culture with lower count shows confluence of 20% (below) c) culture with lower cell count reaches confluence of 70% (below) while the culture with higher cell count has already reached plateau phase (above). d) culture with lower cell count reaches confluence of 100% (below). Culture with higher cell count shows confluence of 100% (above).

## Summary

The zenCELL owl provides fast, accurate and reliable information about the current condition of the observed cell culture by utilizing continual non-invasive long-term analysis. Data about the current cell count and confluence are generated in a fully-automated, objective and reproducible way not requiring any actions on behalf of the user for quality analysis (Figure 7). Besides needing less effort, it also enables users to get more objective and significant information about the status of confluence. Evaluating the current condition of cell cultures is easily possible even online from outside the lab.

Possible uses of the zenCELL owl:

- Documentation and analysis of cell growth
- Determining cell confluence
- Analysing effects of components on cell cultures (cytotoxicity)
- Observation of stem cells
- Migration assays

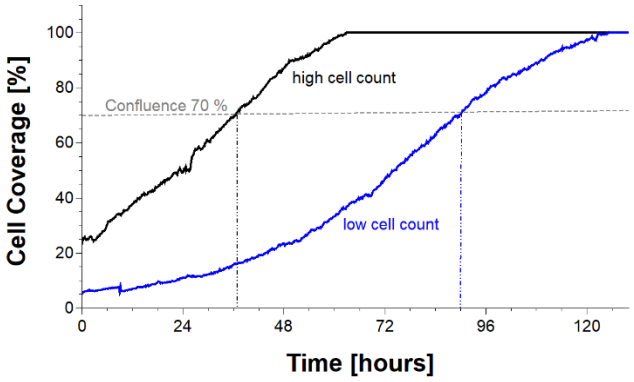



**Figure 7: Analysis of cell confluence performed by zenCELL owl.** zenCELL owl provides an automated, objective and reproducible determination of the point in time at which a specified confluence of the individual wells is reached.