Application Note: Cell migration analyzed with the 24-channel microscope zenCELL owl

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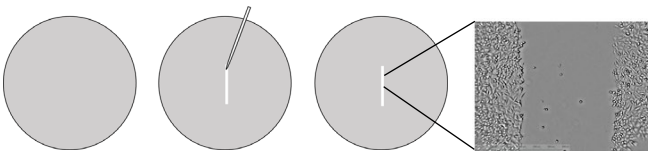
Introduction
Cell migration is involved in many complex physiological and pathological processes. It plays a central role in basic biological events such as tissue generation, renewal and repair, wherein old or damaged cells are replaced by the migration of newly formed cells. Cell migration also occurs in mediating immune responses and wound healing. Undesirable cell migration events take place in the progression of diverse diseases, e.g. cancer invasion and metastasis. For these reasons cell migration has to be controlled carefully to maintain tissue integrity and homeostasis.
A useful tool to analyze cell migration is the wound healing assay. It is a standard method to study cell migration in two dimensions in vitro. A cell-free gap is created in a cell monolayer either by direct manipulation or by physical exclusion. Then the dynamics of the migration into the cell-free area are monitored and quantified. This provides information about cell migration characteristics and wound healing rate. Additional 2D-migration assays can be used for screening the effects of substances for their wound healing and regenerative potential on mammalian cells.
The most common approach to insert a gap in a confluent monolayer is the scratch assay. It uses a pipette tip, needle, or other sharp tool to scratch the cell monolayer. Advantages are the inexpensiveness and easy handling. Another way to create a cell-free gap is physically excluding the cells by using inserts that prevent cells to grow in a defined region. This method generally causes minimal damage to the remaining cells compared to the direct mechanical damage of the monolayer.

In this application note a wound healing assay was performed. Cell migration was imaged using the zenCELL owl incubator microscope (Figure 1). A software especially developed for the zenCELL owl determines 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 current cell count can also be determined. Simultaneously, the microscope documents the quality of each of these individual cell cultures using image recordings. Analyses were performed in all 24 wells of a standard cell culture plate at the same time in intervals of 10 minutes over the measurement period.

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

Cell culture and scratch Assay
L929 mouse fibroblast cell line was seeded in 24-well plates at a density of 120000 cells/well and grown for 24 hours in standard Dulbecco´s Modified Eagle´s Medium (DMEM) supplemented with 5% FBS and 50 µg/ml Gentamycin. Then a scratch (Figure 2) was inserted in the confluent cell monolayer by drawing with a 10 µl pipette tip in each well and rinsed with Phosphate-bufferd saline two times. After addition of fresh cell culture medium the 24 Well cell culture plate was inserted in zenCELL owl microscope and grown for around 24 hours. Incubation environment was maintained at 37°C and a constant 5 % CO_2 in air in a humidified cell incubator. Pictures were taken in intervals of 10 minutes and cell coverage calculated from this values.



a b c

Figure 2: Scratch assay. a) confluent cell monolayer. b) Gap is inserted by a pipet tip. c) Cell-free gap (width 600 µm) before cell migration.

Results:
Microscope pictures show a nearly complete closure of the gap within 24 hours after inserting the scratch (Figure 3). Cells spread and move from the edges of the gap in the free space. Sporadic cell divisions are visible in the pictures. To prevent that cell proliferation could interfere with the measurement of migration, proliferation could be inhibited by addition of drugs like Actinomycin C or deprivation of growth factors (e.g. by serum starving). Data of Cell Coverage correlate with the pictures of wound healing process (Figure 4). Cell Coverage of the area

rises from 50% at the beginning to 90% within 24 hours of the experiment. Calculating the Relative Gap Area (Figure 5) from the Cell Coverage Data allows the determination of the time-point of half gap closure ($t_{1/2}$ Gap Closure).
This Application Note shows the potential of the zenCELL owl microscope for migration assays. The zenCELL owl microscope provides the opportunity to visualize and monitor the migration of cells at every desired time-point of wound healing process. 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.

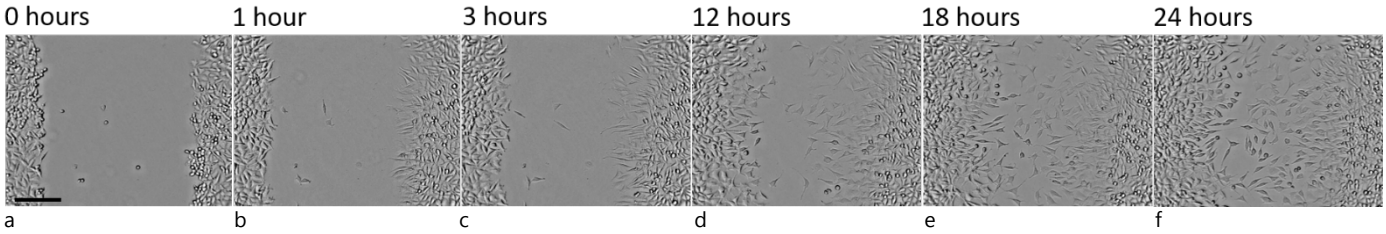


Figure 3: Gap closure over a time period of 24 hours. Images were taken by zenCELL owl microscope. a) The gap was inserted in a confluent monolayer by a pipet tip. b) 1 hour after inserting cells on the edge of the gap start to migrate into the gap. c-e) within a time-period of 24 hours the cell migration result nearly in f) closure of the gap. Scale bar: 200 µm

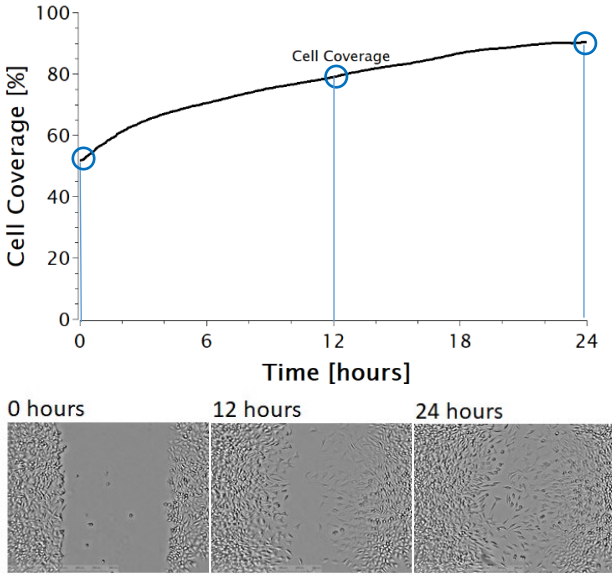


Figure 4: Changes in Cell Coverage during wound healing process. zenCELL owl pictures visualize the progress of wound healing at every point of time.

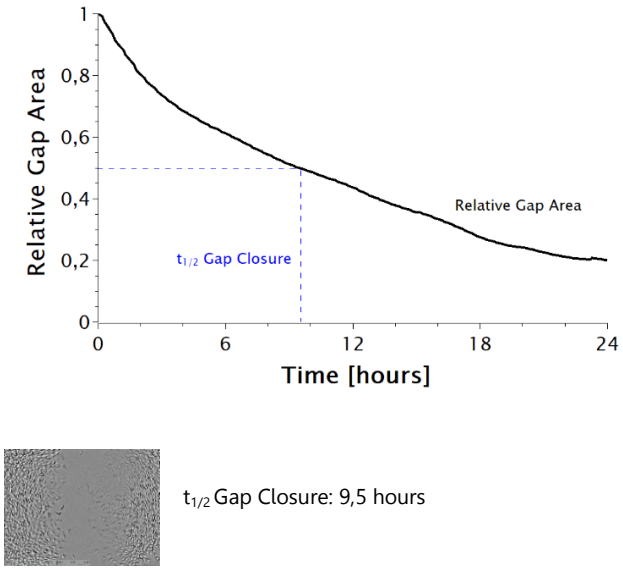


Figure 5: Relative Gap Area. Data of Cell Coverage were used to calculate the Relative Gap Area. Plotted as a function of time, the time-point of half gap closure ($t_{1/2}$ Gap Closure) can be determined from this graph.