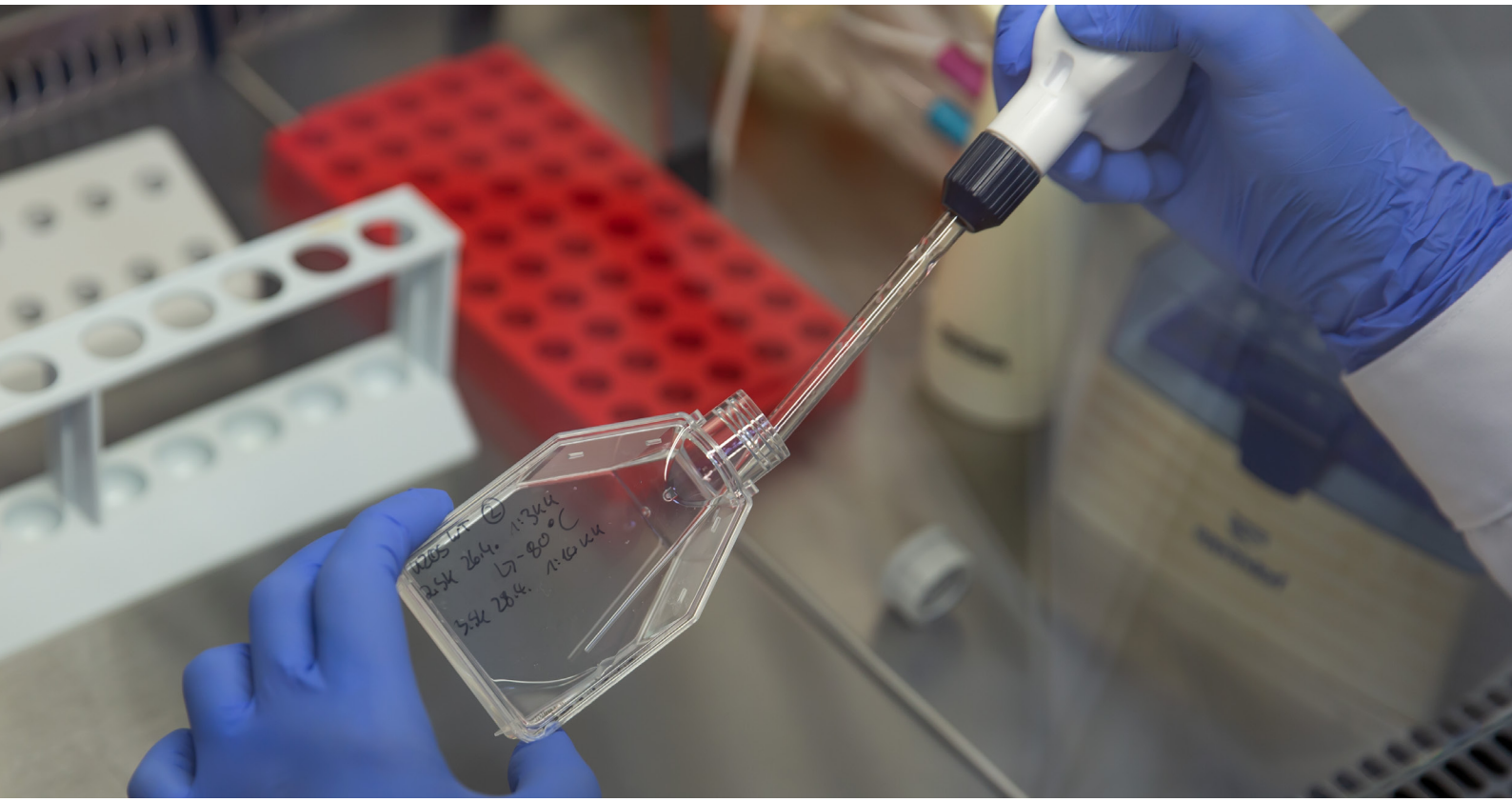
How to standardize cell preparation for experiments

ZEISS Axiovert 5 digital



ZEISS

Seeing beyond

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Cell culture is probably the most important component of a cell biology laboratory. The term covers both – cultivating and replicating eukaryotic cells for further use in experiments. Or, in the case of ZEISS Research Microscopy Solutions, preparing microscopy specimens to use for the development and demonstration of new microscopy techniques dedicated to the life science and biomedical industry. Working in our fully equipped biomedical laboratories – experienced ZEISS technicians deal with all everyday issues, questions, aspects concerning cell culture. Each cell type must be cared for according to its individual needs and all cells must be checked on a daily basis. This involves measuring the cell density, determinating the cell count as precise as possible and handling cells for sample preparation. At ZEISS Research Microscopy Solutions this work is done using the inverted microscope ZEISS Axiovert 5 digital using Artificial Intelligence.

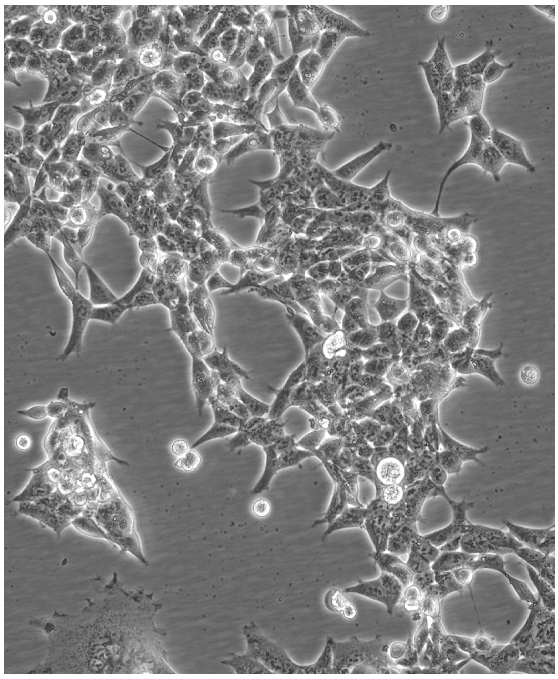


Figure 1 *HeLa cells; phase contrast, objective: LD A-Plan 20×, acquired with ZEISS Axiovert 5 digital*

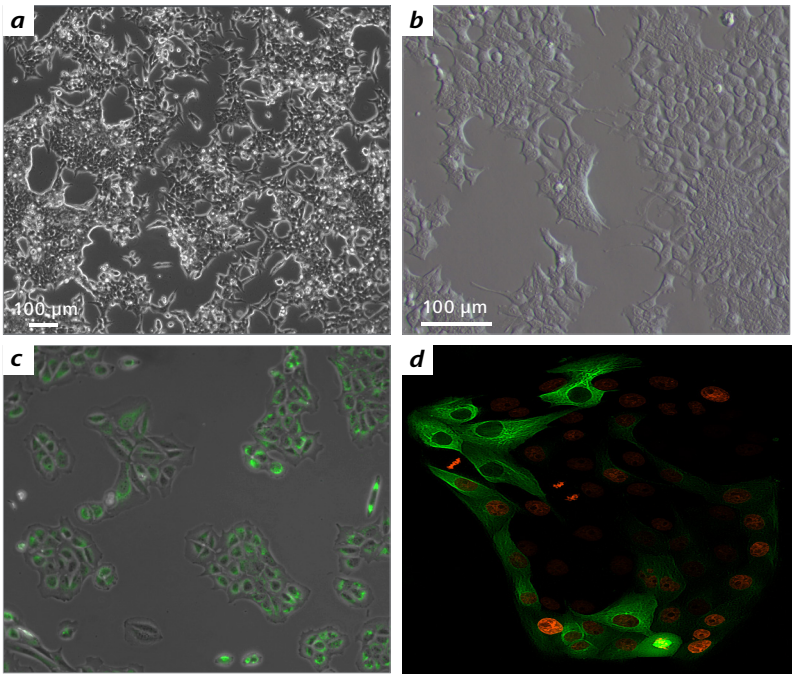


Figure 2 *a) HEK cells, phase contrast, objective: LD A-Plan 10×, acquired with ZEISS Axiovert 5 digital; b) Cos7 cells, iHMC contrast, acquired with ZEISS Axiovert 5; c) U2OS cells, tranfected with MitoTracker green, overlayed with phase contrast, objective: LD A-Plan 10×, acquired with ZEISS Axiovert 5 digital; d) LLC-PK1 cells, cell nucleus – mCherry, EGFP – Alpha Tubulin, objective: Plan-Apochromat 20×, acquired with ZEISS Axiovert 5 digital*

Cell culture and cell lines

To bring cells into culture, you can either cultivate cells from a primary donor or introduce tiny quantities of a specific cell line from an external source. Typical cell lines used in the ZEISS microscopy laboratory are immortalized epithelial or cancer cells such as HeLa, HEK, COS-7, U2OS and LLC-PK1.

Each cell line has characteristic properties such as size, appearance, or strength of attachment. The speed of propagation is also different in all individual cultures.

Figure 3 *Cells are kept in incubators with defined conditions.*

Task

Prepare an LLC-PK1 cell culture for an application study

Preparing a culture from frozen cells

LLC-PK1 cells were thawed in a water bath at 37 °C and are transferred to a sterile test tube containing cell culture medium. The cells are then centrifuged for approx. five minutes with 900 rpm.

LLC-PK1 refers to pig kidney epithelial cells. This cell line, derived from the kidney of a normal, healthy male pig (*Sus scrofa*), aged 3 – 4 weeks was deposited by Eli Lilly & Co. and exhibits typical epithelial morphology.

The supernatant first needs to be discarded / aspirated. The pellet or parts of it can then be resuspended and transferred in a T75 culture flask with approx. 10 ml of culture medium. The culture flask is now placed in the incubator at 37 °C, 5 % CO_2 and a relative air humidity of 97 %. The culture medium will then be exchanged with new medium after 24 h of incubation.

Figure 4 *Different cell lines and culture vessels in the incubator*

Health check and contaminations

Cell growth and morphology are monitored daily using phase contrast microscopy. Various contaminations can occur when working with cell cultures – most commonly, moulds, yeasts and bacteria. A particularly unfavorable type of bacterial contamination may be caused by mycoplasma. Compared to other bacteria, these very small procaryotic organisms lack a cell wall and are hard to detect.

Morphological irregularities and the color (Figure 6) of the culture media can be a first indication of an unhealthy cell culture.

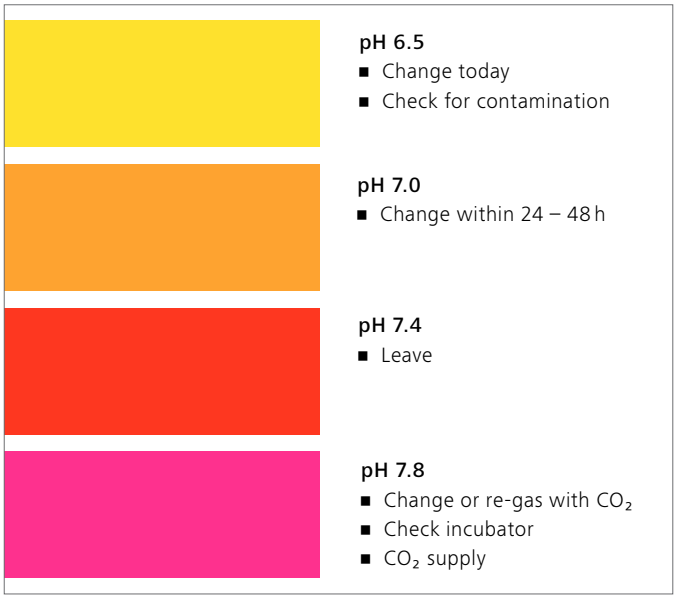


Figure 5 *Culture medium color is indicating pH value*

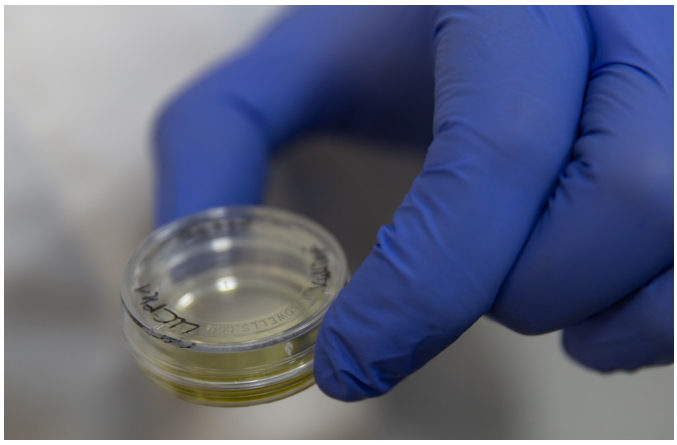


Figure 6 *The yellowish medium of this LLC-PK1 cell culture indicates, that the pH is too low (acidic).*

If no morphological peculiarities or contaminations are detected the cells are further cultivated in cell culture flasks until they reach the density (confluency) specific to their cell lines needs for splitting. Depending on the cell line and medium used, growth rates can differ.

For most of the commonly used cell lines, you will need to reach a confluency of 70 – 80 % before the cells are ready to be split and passaged for further experiments. This is especially important as you will want to avoid unnecessary work caused from splitting the cells too early. Splitting the cells at a specific confluency is essential for the outcome of the experiments and their reproducibility. Splitting to early or to late can lead to uncharacteristic cell behaviour or reduced culture growth.

Using the cell imaging microscope Axiovert 5 digital to determine cell confluency and cell count is very easy. AI algorithms help to work faster and more reproducible. The software modules especially designed for these tasks are easy to use without prior knowledge in Artifical Intelligence. They provide higher quality standards for every cell culture lab.

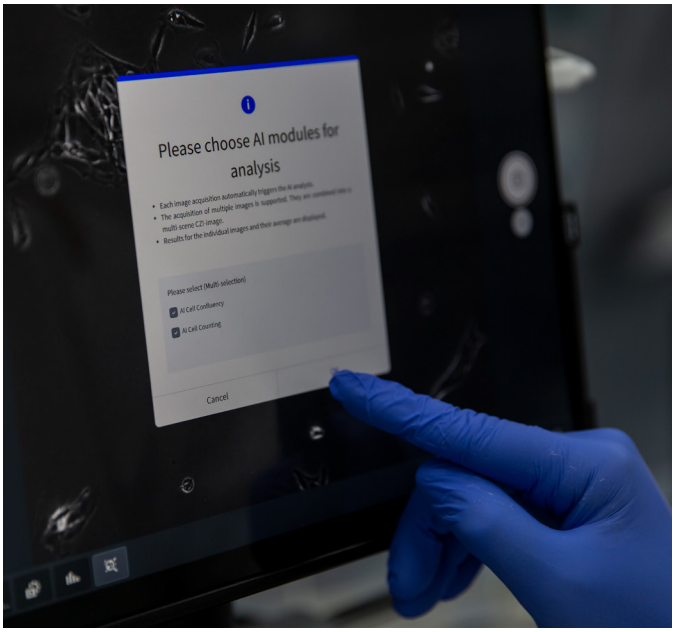


Figure 7 *Artificial Intelligence based software helps measure confluency of cells.*

Apart from the daily cell culture routine, cell confluency and cell count are also important parameters to define the conditions in an experiment dish, to evaluate end point assays, and to optimize cell transfection protocols. With the AI modules in Labscope this task becomes simple.

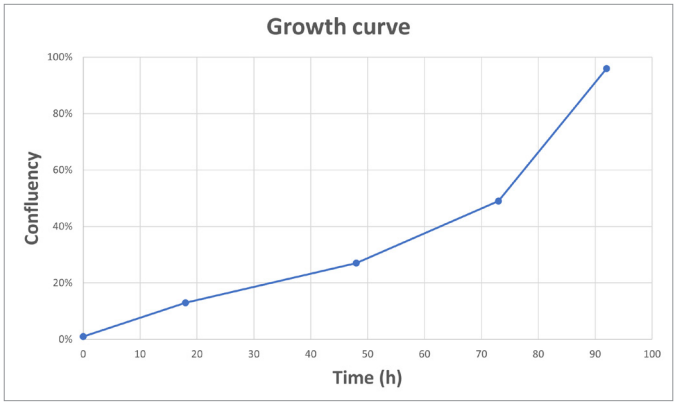


Figure 9 *Growth curve*

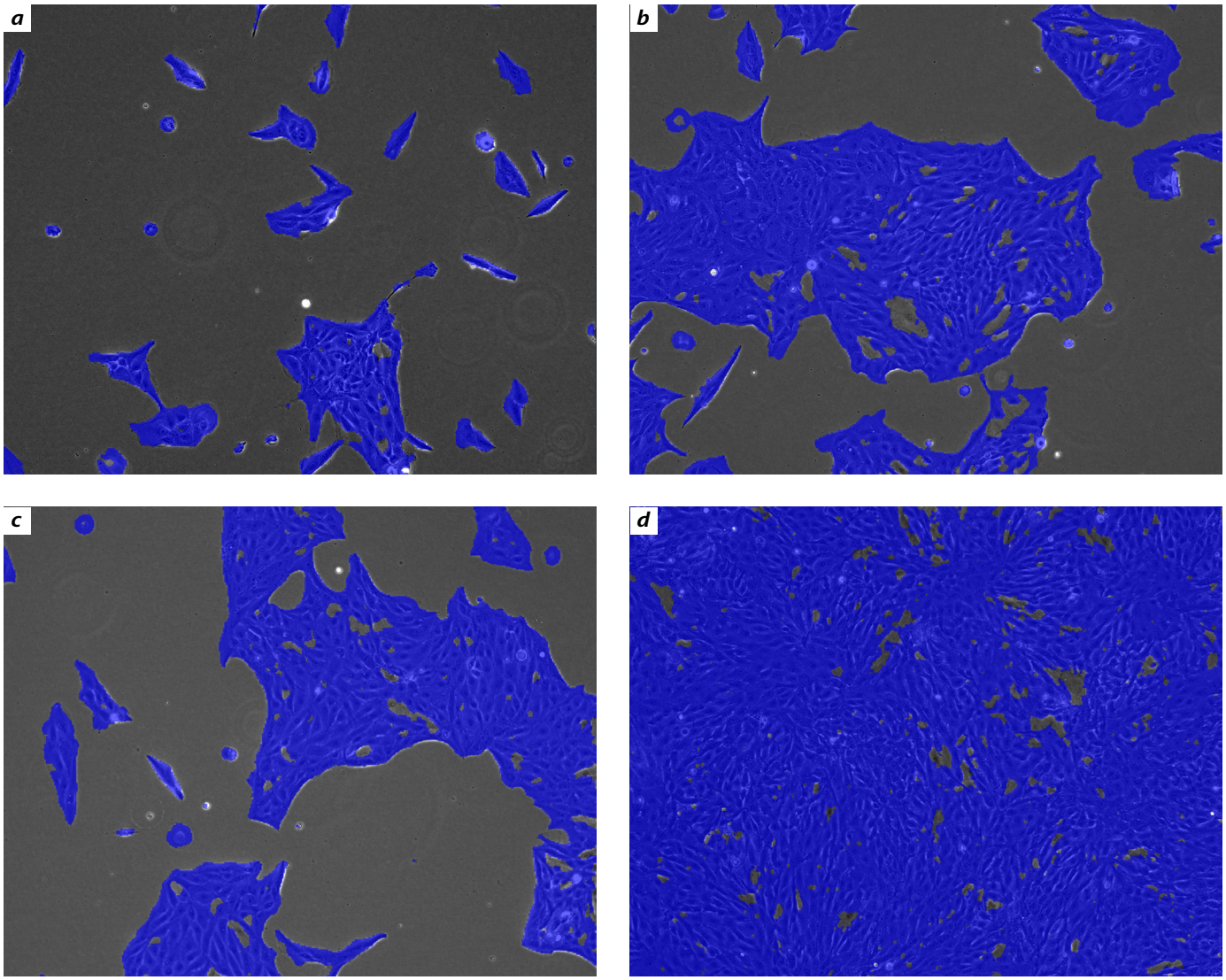
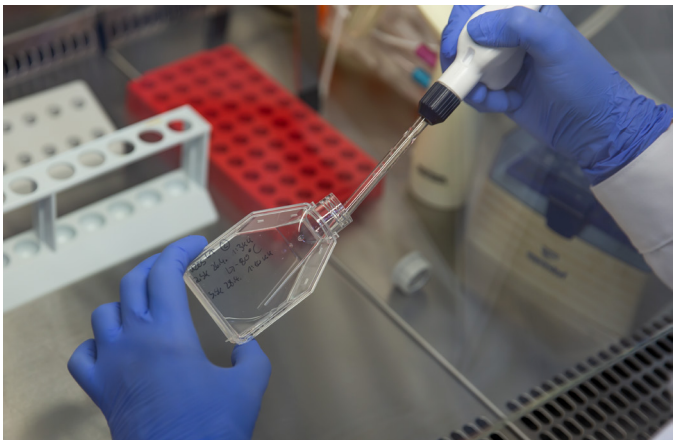
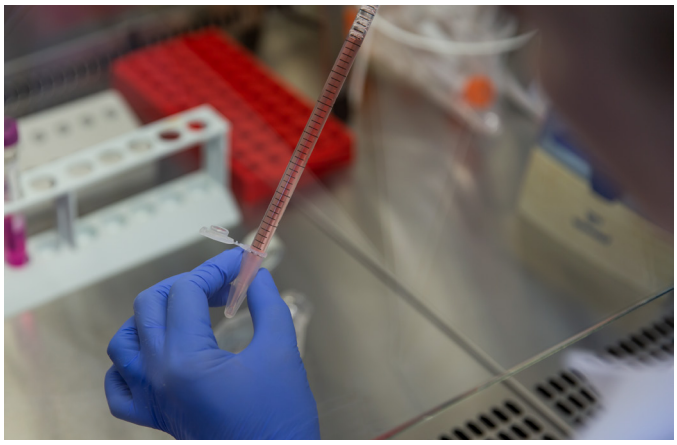


Figure 8 *Cell confluency observed at different time points*

Subcultivation

Once the appropriate confluence level in the culture vessel has been reached, the cells must be divided into different cell containers according to the number of cells needed for the planned experiment (Figure 10). Each cell line has its specific number of cells that need to be seeded in a certain volume of medium. At first, adherent cells must be detached from the bottom surface of the flask by using trypsin or mechanical force. After detachment they are brought into suspension with a defined volume of medium. By measuring a small aliquot of the suspension, one can determine the cell concentration using a microscope and phase contrast. The correct volume of suspension is then distributed into different cell culture flasks and the cells are topped with fresh growth medium and placed in an incubator to grow.





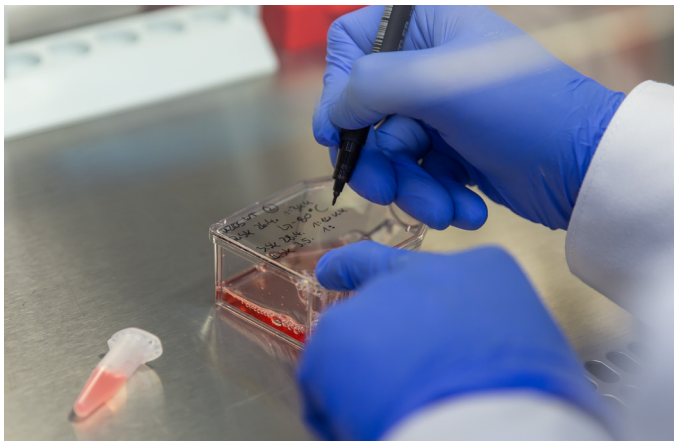


Figure 10 *Splitting and passaging LLC-PK1 cells*

Traditionally this time consuming work is done manually using a haemocytometer (e.g. improved Neubauer counting chamber). The cells in the 4 corner squares (each is 1 mm × 1 mm) can be counted in phase contrast. The concentration (MV) or total number of cells per ml is calculated via: MV = [(Total number of cells counted in 4 squares / 4) × dilution factor × chamber constant] per ml. A typical chamber constant is 10^4. Depending on the desired accuracy, less than 4 squares are sometimes counted.

Determining the vitality of the cells

If you are about to characterize the conditions in your dish for an experiment it is no longer necessary to detach the cells for cell counting.

Now, it's easier and, above all, more cell-friendly to leave this procedure to the AI Cell Counting Module from ZEISS Labscope. Automatic cell counting of the cells in each batch of cells is carried out in seconds by means of phase contrast microscopy and without bringing the cells out of their growth phase. Thus, you can measure the cell count in a field of view with just one click. Repeating this at multiple positions in your sample will result in an averaged and extrapolated total cell count of this cell culture vessel.

Working with this module allows for a reproducible cell count and confluency measurement, directly in your culture vessel. It is fast and fits in the overall cell culture routine workflow. The module can improve the quality of your routine cell culture without additional work.

A fast measurement of cell count and cell confluency is also recommended before doing experiments. Cell response can depend strongly on cell-cell interactions. Therefore, cell number and confluence are important parameters to characterize the experiment conditions in advance. This allows you to exclude unwanted variations and to perform the experiments in a more reproducible way.

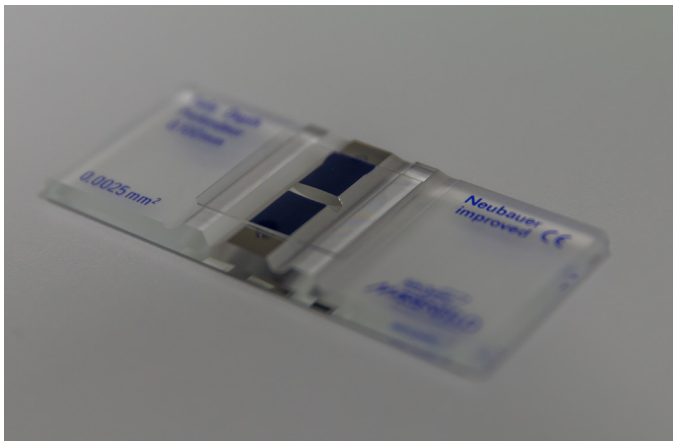


Figure 11 *Neubauer improved hemocytometer*

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