fluidlab R-300

Using the histogram correctly

When analysing cell count and viability of cell cultures with the fluidlab R-300, the histogram is an essential part of the result presentation. The histogram shows the size distribution of all detected cells in the cell culture. It is a bar chart in which the height of each bar indicates the number of cells within a certain size interval. A high bar thus shows that many cells with a similar cell size were detected. The histogram contains information about the average cell size as well as the distribution of the cell size in the cell suspension.

Fig. 1.: Result screen of the fluidlab R-300 Cell Counter with histogram and microscope image

How to use the histogram?

Setting the size interval

At the beginning of a measurement the expected size interval of the cell culture sample can be set. We **recommend to initially consider the whole size interval** in order to detect all cells in the cell culture. For cell counting, cells from 3 - 80 µm can be detected and for viability analysis cells in the range of 8 - 80 µm can be detected. The size interval can be further adjusted in the result screen after the measurement.

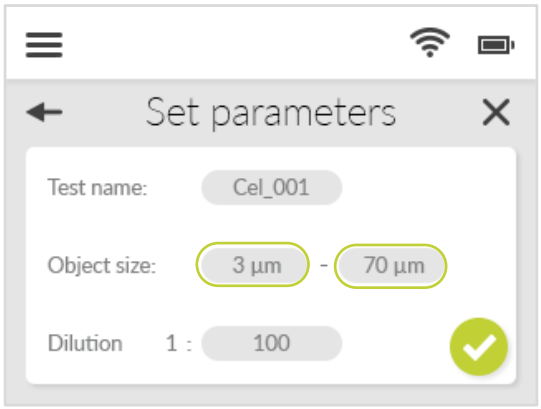

Fig. 2.: Setting the size interval before measurement

Adjusting the Gatings

The result of the measurement is given in cells/ml for the selected cell size interval. In the middle part of the result screen, you can now view the histogram. The vertical lines correspond to the preset upper and lower limits of the cell size. By moving these lines, you can easily adjust the size interval as required, e.g. to exclude debris or to view only a specific cell population. The numerical values of the lower and upper limits can be read directly above the histogram. Alternatively, the size interval can also be adjusted by manual input in the grey shaded fields. By limiting the size interval, the so-called gating changes accordingly, i.e. only cells within the set size interval are counted.

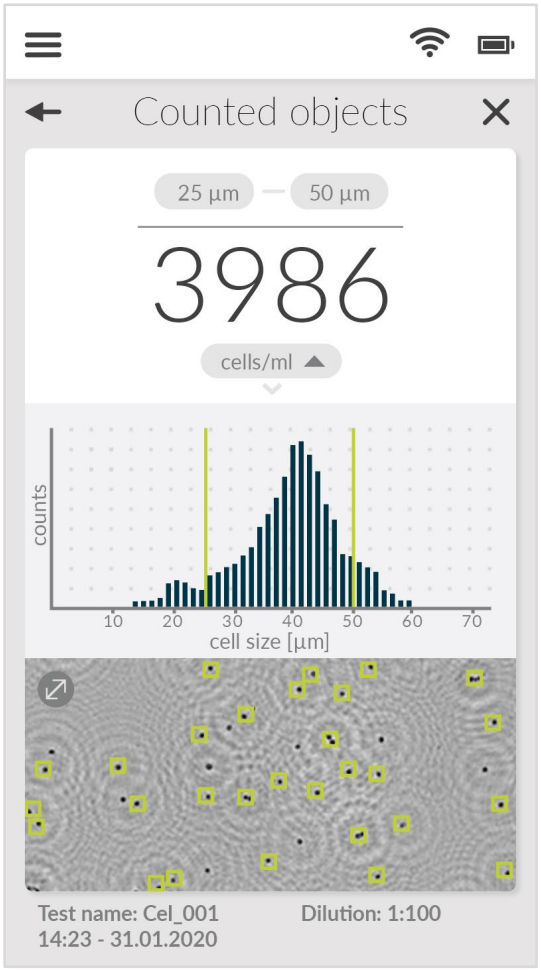

Fig. 3.: Setting of the gating and result

Effects on the result

An adjustment of the gating in the histogram has direct influence on the result of the cell count and viability determination. If the size interval in the histogram is shifted, the cell count in the result screen above changes dynamically. We recommend always checking the histogram and the set gating before reading the cell count or viability values.

What additional information does the histogram give me?

How large are my cells on average?

In a homogeneous cell population with small variations in cell size, the histogram approaches a bell-shaped curve with a clearly defined maximum. This maximum in size distribution corresponds to the average cell size.

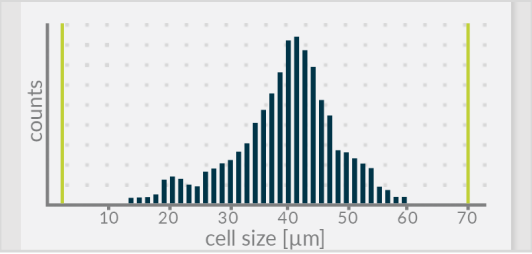

Fig. 4.: Homogeneous cell population

Is my sample contaminated?

Outliers in the histogram can indicate sample contamination or cell clumps. Thus, the histogram contains valuable information to check the quality of the cell culture.

Are the cell populations of my sample different?

Heterogeneous samples of different cell sizes can be recognized in the histogram by the occurrence of several maxima as well as a broad size distribution. By appropriate gating, the different cell populations can be analysed separately.

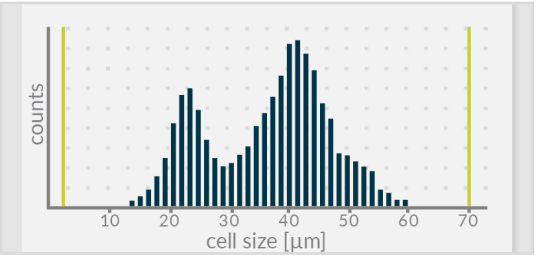

Fig. 4.: Heterogeneous cell population