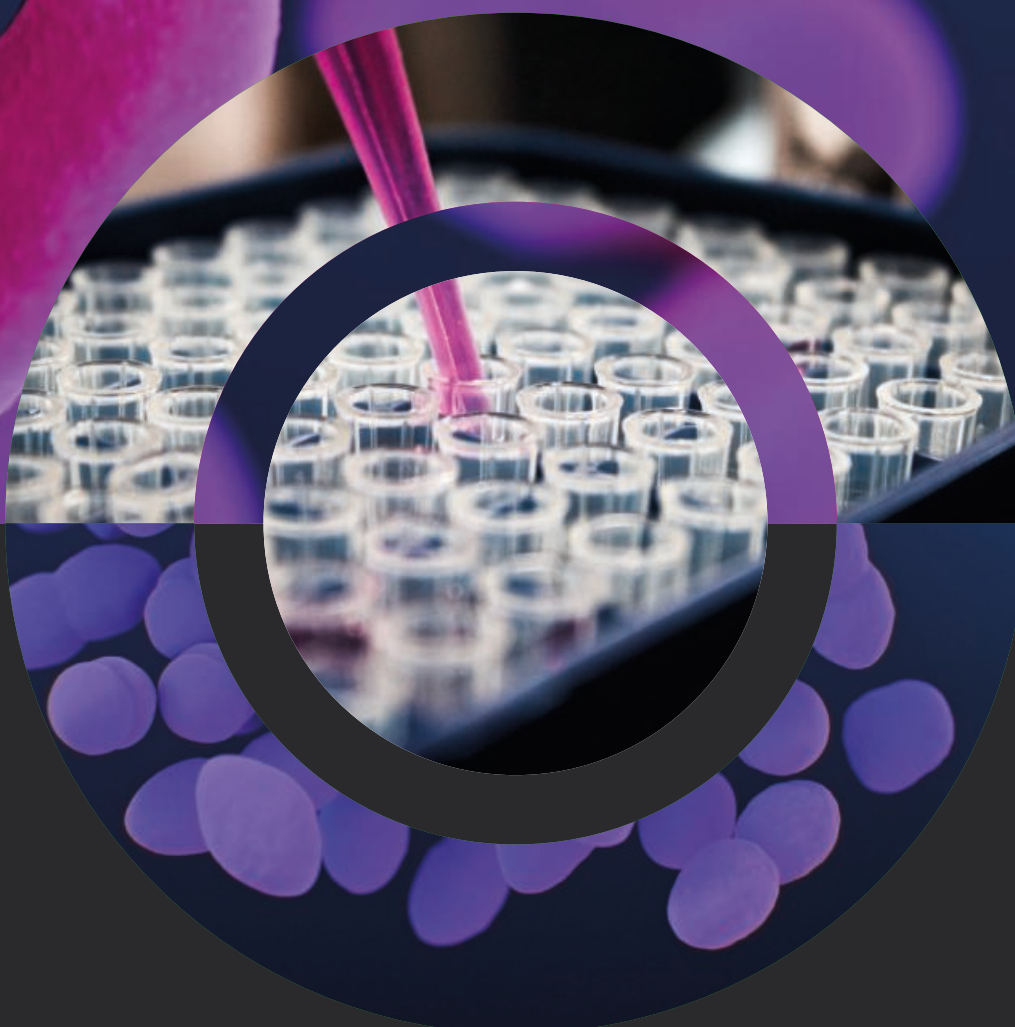




SHARED RESOURCES LABS

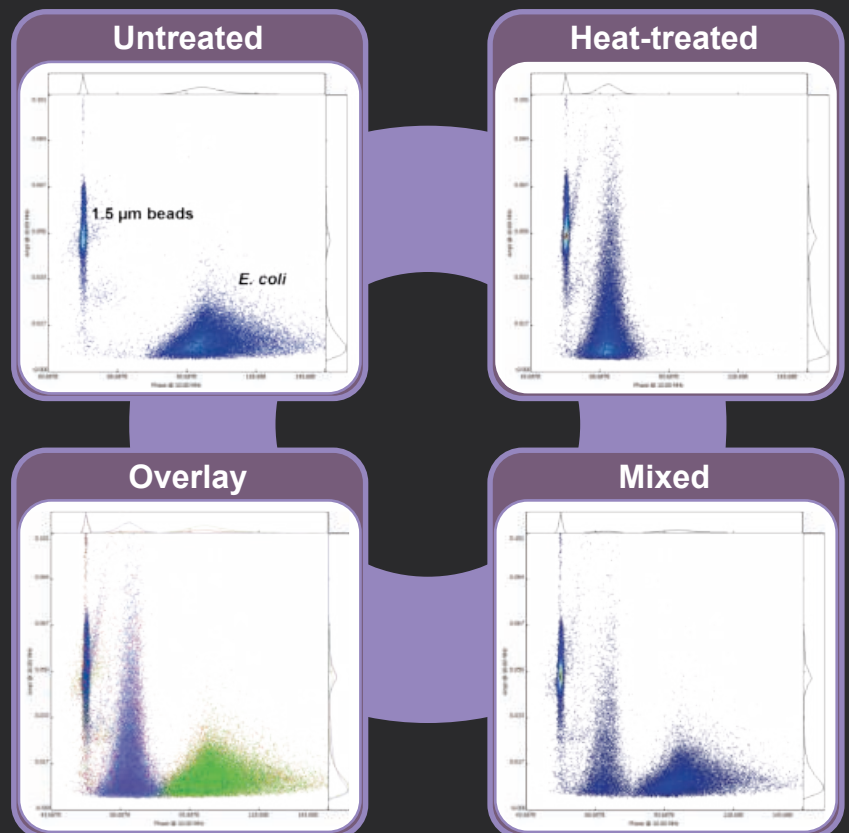
ACADEMIC AND INDUSTRIAL RESEARCH

Close the gap between simple cell counters and highly sophisticated flow cytometers: Impedance Flow Cytometry offers single-cell analysis in a fast and easy way for determination of cell count, viability and metabolic status – simultaneously!

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COUNTING AND VIABILITY OF BACTERIA

Measuring the number and viability of bacterial cells presents significant challenges due to the microscopic size and diverse nature of bacteria. Traditional methods, such as viable plate counts, can be time-consuming and may not detect all viable cells. Advanced techniques like flow cytometry and molecular methods offer more precision but require specialized (and expensive) equipment and expertise. Accurate bacterial counting is crucial for various applications, yet viability analysis is relevant as well. Amphasys' technology enables accurate counting and viability assessment of bacteria within a minute – without the need for calibration or incubation. An asset not only in antibiotic research!



The figures illustrate measurements of an over night culture of *E. coli* in LB medium. Samples are spiked with 1.5 μm beads for reference. Measurements were conducted using Amphasys' proprietary buffer.

The untreated culture shows only viable *E. coli* cells. Heat treatment at 99°C for 30 minutes results in only dead cells. When untreated and heat-treated samples are mixed in a 1:1 ratio, the mixed sample clearly shows both bacterial populations separated. The same result is observed when overlaying scatterplots of the untreated and heat-treated samples. Measurements of cell count and viability are carried out in parallel, without any need for calibration.

YEAST FERMENTATION

You start a fermentation with yeast cells and measure a viability of 75%. Do you know if the cells are dormant or in the early exponential phase already?

Figure 1

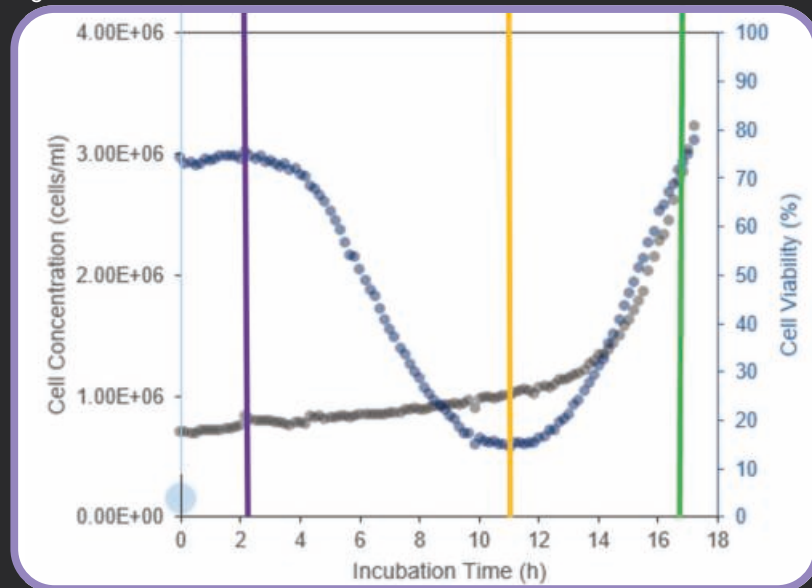


Figure 1 illustrates a time-resolved measurement of yeast cell concentration and viability measured over 17 hours in an online set-up and shows the behaviour of the yeast cells. The grey dotted line indicates cell concentration during the lag and early exponential phases, while the blue dotted line represents cell viability. Figures 2a-c display corresponding scatterplots at 2.5 hours, 11 hours, and 16.5 hours. The red line in each scatterplot separates dead cells (left) from viable cells (right).

While a biomass sensor displays the growth in cell concentration, Impedance Flow Cytometry in addition shows the exact viability and the status of the cells. Figure 2a shows after 2.5 hours dormant yeast cells at 75% viability. These cells are going to die in the course of the next hours, represented by the increase in dead cells in figure 2b, only leaving a small concentration of viable cells. After 11 hours the exponential growth starts, represented by the large population of viable cells in figure 2c.

Amphasys' technology provides much more information beyond cell count and viability and helps to develop, optimize, and control bioprocesses.

Figure 2a

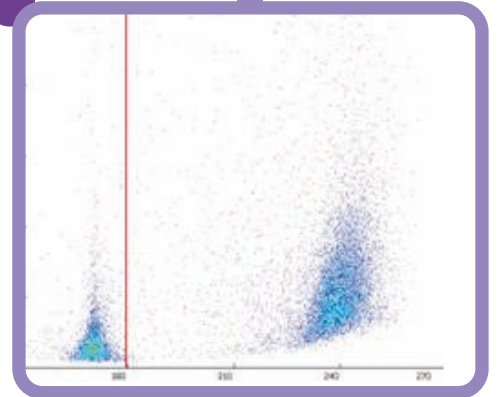


Figure 2b

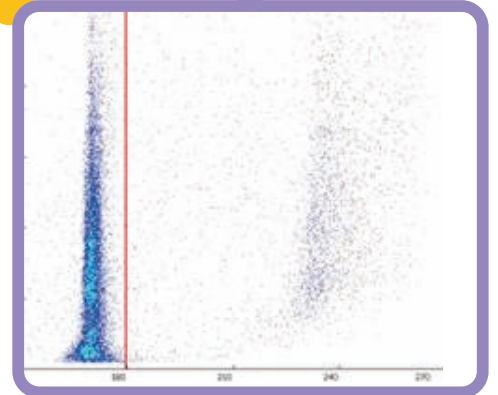
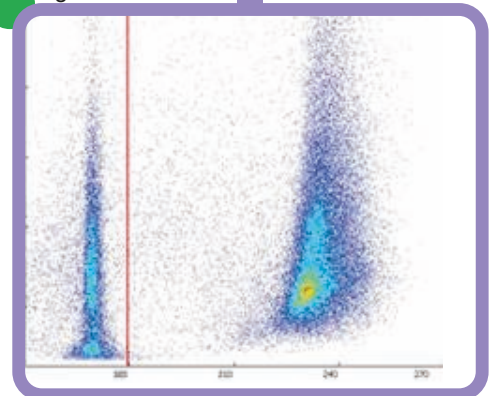


Figure 2c



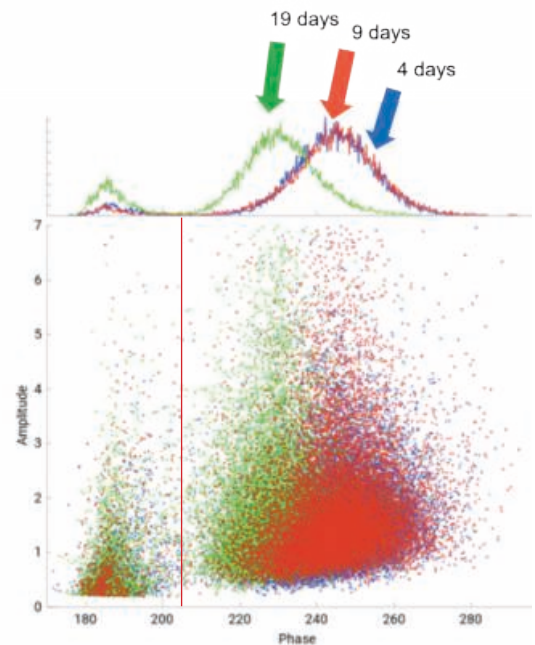
METABOLIC STATUS MONITORING

DO YOU KNOW
HOW YOUR CELLS
ARE DOING?

The adjacent figure illustrates the monitoring of adherent CHO cells over 19 days. Three overlaid scatterplots show the status after 4 days (blue), 9 days (red), and 19 days (green). Dead cells are located left of the red line, viable cell populations are spotted right to the red line.

- Healthy status: in the first 9 days cells are viable with sufficient nutrients. The histogram above the scatterplot shows a complete overlap of the two curves.
- Starving status: the green population shifts towards dead cells. The histogram clearly shows this shift together with an increase in population of dead cells.

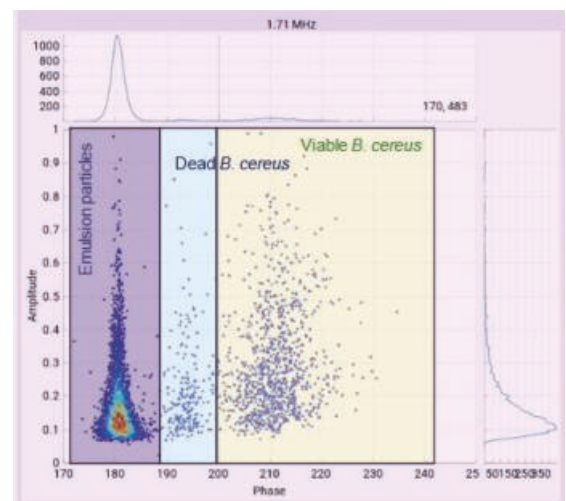
The depletion of nutrients leaves the cells starving which causes changes in the membrane integrity of the cells. Amphasys' technology detects these change in the membrane and provides indications to the health status of the cells.



CELL ANALYSIS IN MATRICES

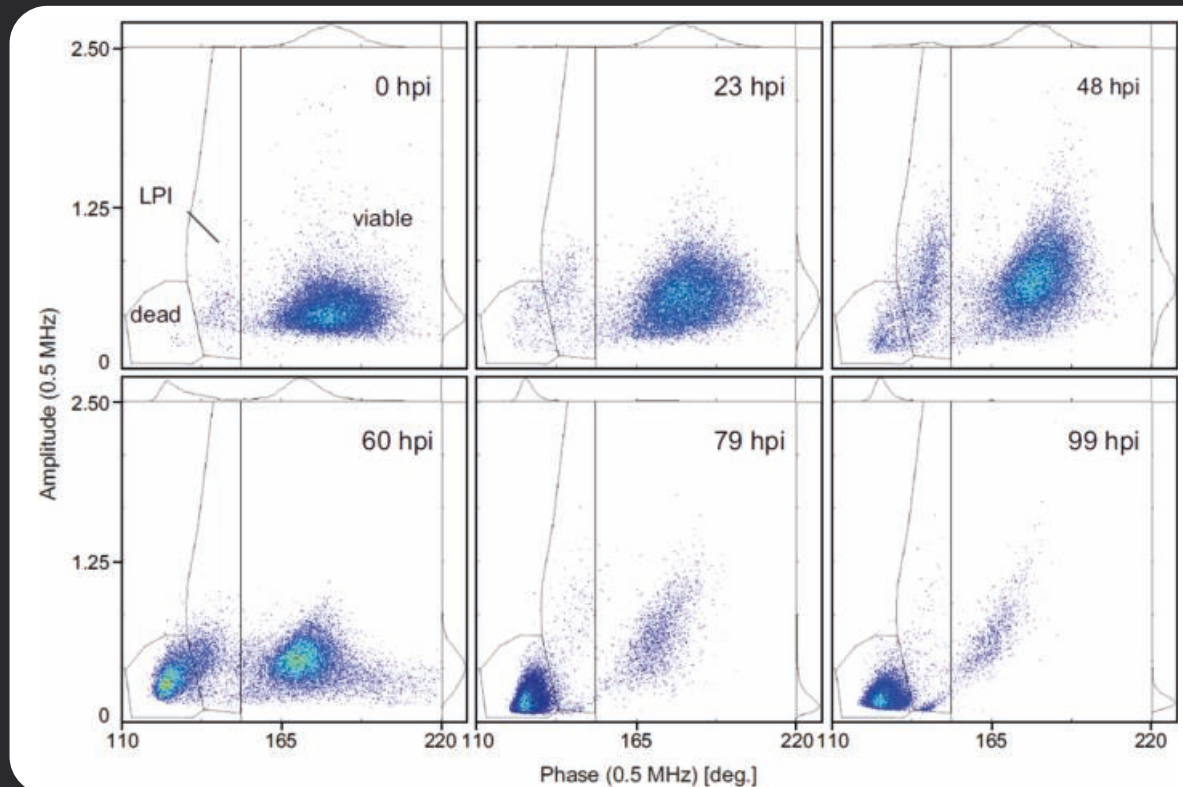
Turbid, opaques, and fluorescent media present challenges for systems with optical detection. Impedance Flow Cytometry is based on electrical detection and not affected by the opacity of the medium. This allows for cell analysis in complex and opaque matrices, such as milk or polymer emulsions as well as in autofluorescent media of microalgae.

The figure illustrates the measurement of a sample consisting of an aqueous emulsion of a synthetic polymer which is contaminated with *Bacillus cereus*. The scatterplot shows the clearly defined polymer particles at the left. In addition, the separated dead and viable bacterial cells are displayed. An exact quantification of all particles and cells is given by default.



PROTEIN EXPRESSION IN INSECT CELLS

Metabolic activity and process control: Impedance Flow Cytometry allows to detect protein expression in cells and to determine the right time for harvesting in the bioprocess.

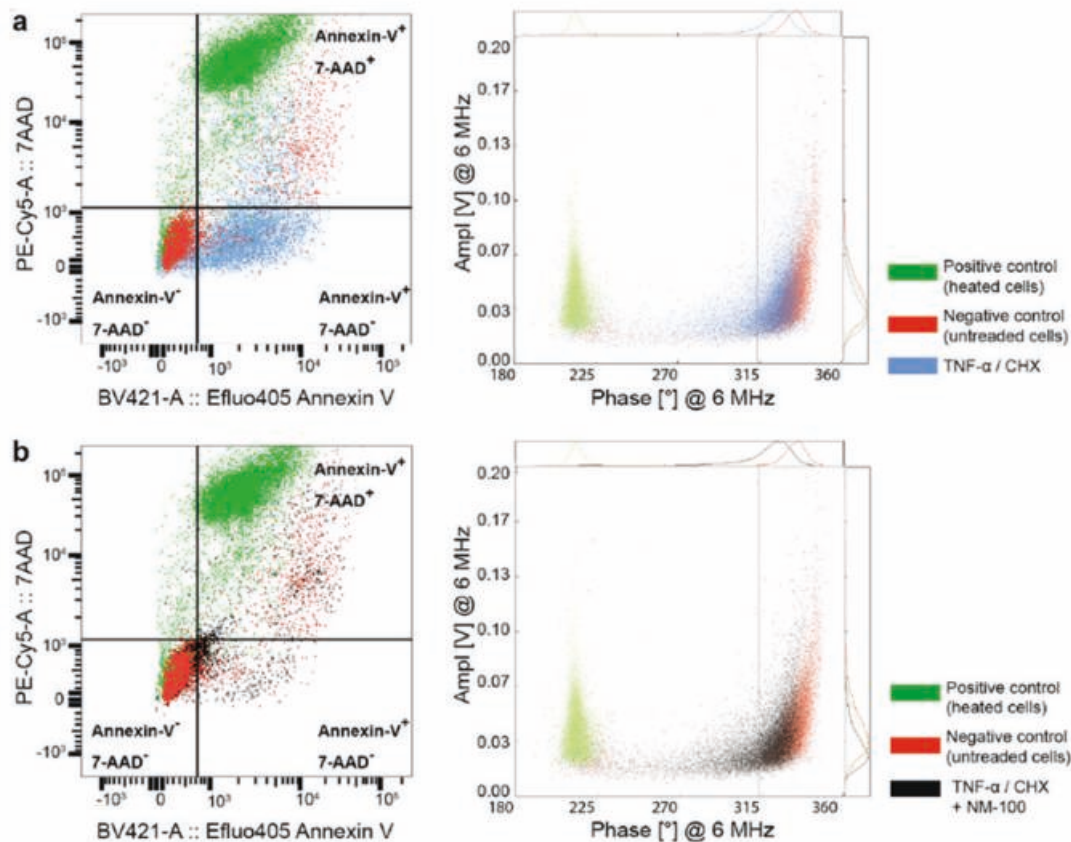


The above scatterplots illustrate the protein expression over time in Sf9 cells, which were infected with Baculovirus.

- Viable population: in the first 48 hours after infection the population of viable cells shifts to the upper right, indicating an increase in cell size caused by the viral load. In the later stages at the end, this viable population nearly completely disappears.
- Dead population: the population of dead cells constantly increases, especially from 60 hours post-infection onwards.
- LPI population: a third population can be detected which represents cells in the late phase of infection. They appear between 48 and 60 hrs after infection and the cell concentration correlates with the concentration of the protein. The disappearance of this population at 79 hours indicates complete lysis of the cells and release of the protein in the supernatant.

Amphasys' technology allows to monitor metabolic activity of cells and control bioprocesses in an effective way.

NANOTOXICITY MONITORING



The advantage of label-free cell analysis is shown in this comparative study where nanoparticles prevent markers to adhere to the cell wall. The figure compares conventional flow cytometry (left) with impedance flow cytometry (right). U937 human lymphoma cells were exposed to TNF/CHX to induce apoptosis, both without TiO₂ nanoparticles (top row) and with TiO₂ nanoparticles (bottom row).

- Without Nanoparticles: Red viable cells, green dead cells, and blue apoptotic cells (TNF/CHX treated) are visible in both scatterplots of conventional (left) and impedance flow cytometry (right).
- With Nanoparticles: the apoptotic cells are not detected by means of conventional flow cytometry (bottom left) as nanoparticles prevent the marker to adhere to the cell wall. Impedance flow cytometry detects the apoptotic cells (black) without interference.

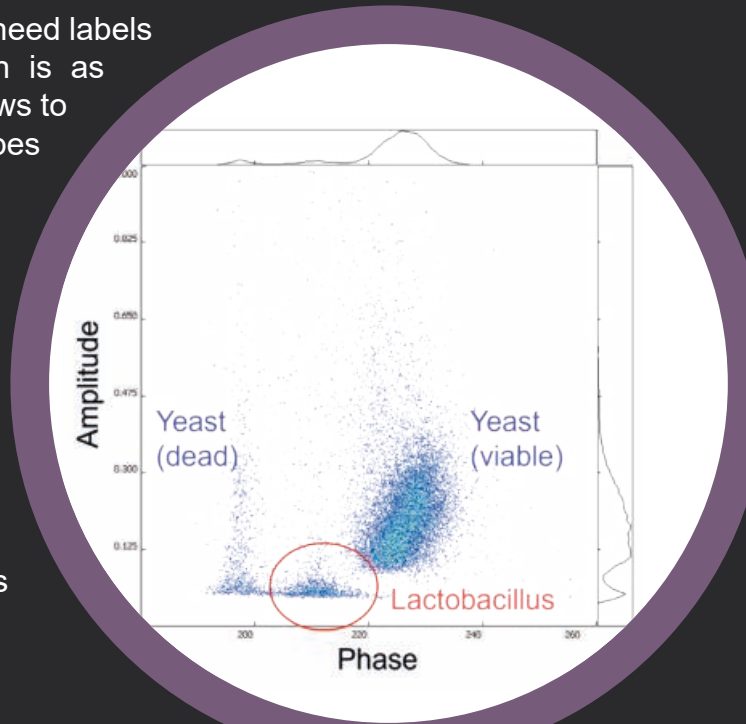
Label-free impedance flow cytometry offers advantages in these cases where adhesion of markers is blocked or where markers are not available.

CONTAMINATION: MEASUREMENT OF TWO CELL TYPES IN PARALLEL

Impedance Flow Cytometry does not need labels or markers, and sample preparation is as easy as diluting and filtering. This allows to detect contaminations of other cell types which are of smaller or similar size.

The figure illustrates a yeast culture separated into a viable and a dead population. In addition, a contamination of smaller, viable *Lactobacillus* cells is detected.

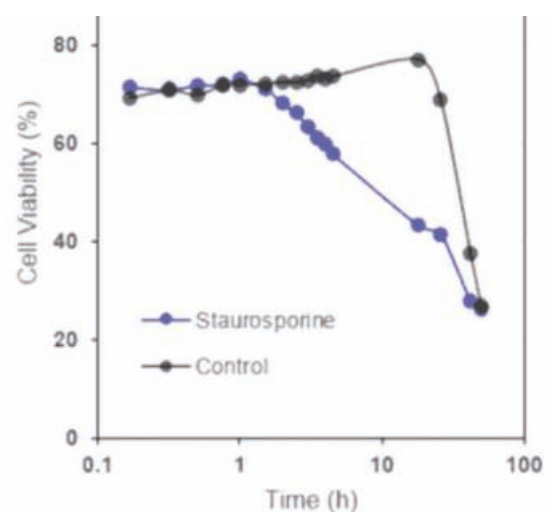
Amphasys' impedance-based technology enables simultaneous measurement of cells and particles of similar size, and benefits from the fact that no specific markers or labels are needed.



APOPTOSIS MEASUREMENT

Amphasys' impedance flow cytometry is a very fast method for the characterization of cells. Not only sample preparation requires a minimum of time, but also measurements are done within seconds. Results are obtained real-time as no incubation is needed.

The figure shows time-resolved viability measurements of Burkitt lymphoma cells after treatment with staurosporine (blue line). Cell viabilities of an untreated reference sample (black line) were measured in parallel.



The decline in viability of the treated sample can be observed in real-time, without any delay, and as early as one hour after the treatment the decline in viability already starts (note: logarithmic scale). Similar measurements were done to show the efficacy of antibiotic treatments on bacteria.

NEW ERA OF CELL ANALYSIS

Ampha X30



Experience simultaneous, label-free measurement of cell count, viability, and metabolic activity. Assess the whole range of cells: bacteria, algae, yeast, human and animal cells without the need for markers, dyes, incubation, or calibration.

Powered by Impedance Flow Cytometry, the Ampha X30 is the universal benchtop instrument for cell analysis of any organism. Sophisticated software facilitates to uncover the hidden secrets.

LABEL-FREE ANALYSIS

No staining, no markers, no apoptosis kits necessary. No incubation nor calibration.

DIFFICULT MEDIA

Unaffected by turbidity, opacity, autofluorescence, and particles. Measures somatic cells in milk and contaminations of different cell types.

COMPREHENSIVE ANALYSIS

All cell types from 1 to 50 μm . Cell count and viability simultaneously. Cell health status and metabolism as a bonus.

COST-EFFECTIVE and FAST

Low operational and investment costs. Very short sample preparation and measurement times.

READY TO ELEVATE YOUR RESEARCH?



The information contained herein is to the best of our knowledge and complete. However, measurement samples and cell species vary in properties. Therefore, deviation to other cell analysis methods may occur. Nothing contained or stated herein, including results obtained from the use of Ampha X30, shall be construed to imply any warranty or guarantee.

Amphasys AG shall not be held liable for damages, and customers shall indemnify Amphasys AG against liability resulting from the use of potentially inaccurately generated data with Ampha X30. It is recommended that all results obtained with Ampha X30 be validated against appropriate reference methods at regular intervals.