APPLICATION NOTE

CHARACTERIZATION OF CANCER CELLS BY IMPEDANCE FLOW CYTOMETRY

Silvan Kaufmann, Grit Schade and Marco Di Berardino, Amphasys AG

INTRODUCTION

Impedance Flow Cytometry (IFC) is a technique to rapidly characterize a large amount of cells at the single-cell level [1,2]. The readout depends solely on the electric properties of cells, thus no labelling is required and sample preparation is minimal. This makes IFC exceptionally well-suited for applications that require a quick time-to-result, field measurements and in-line analytics.

Here, we report characterizations of BL2 cell cultures (Burkitt lymphoma origin) under various conditions. Using Amphasys IFC technology, cell viability and concentration can easily be determined. In addition, we show that cells undergoing apoptosis lead to a distinct impedance response.

SUMMARY

- Label-free and rapid characterization of large numbers of BL2 lymphoma cells at the single-cell level
- Clear distinction between viable and dead cells due to large differences in impedance phase
- Cell viability and concentration can be monitored over time
- After induction of apoptosis, viable cells show a pronounced phase shift. Impedance phase was identified as a valuable predictor of cell state.

IMPEDANCE FLOW CYTOMETRY

The Ampha Z32 flow cytometer is a benchtop instrument, equipped with a microfluidic - microelectronic chip. Cells in suspension are pumped through the microchip, where they are exposed to an alternating current electric field. The passage of a cell through the electric field alters the measured impedance signal, depending on the cell's electric properties. Multiparametric characterization of cells is achieved by the application of up to 4 frequencies simultaneously.

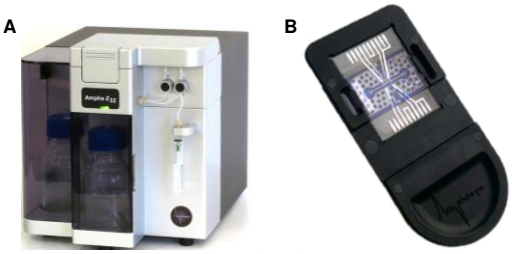

Figure 1: A) Amphasys Ampha Z32 Impedance Flow Cytometer B) Amphasys Measurement Chip

The impedance raw signal, consisting of a real and an imaginary part (Fig. 2 B), is processed by the cytometer electronics and impedance data is visualized almost real-time using the AmphaSoft software. Data is displayed in Phase – Amplitude scatterplots (Fig. 3). Each data point corresponds to the impedance response of one measured cell. Depending on the cell concentration of the sample, several hundreds to more than a thousand cells can be analyzed per second.

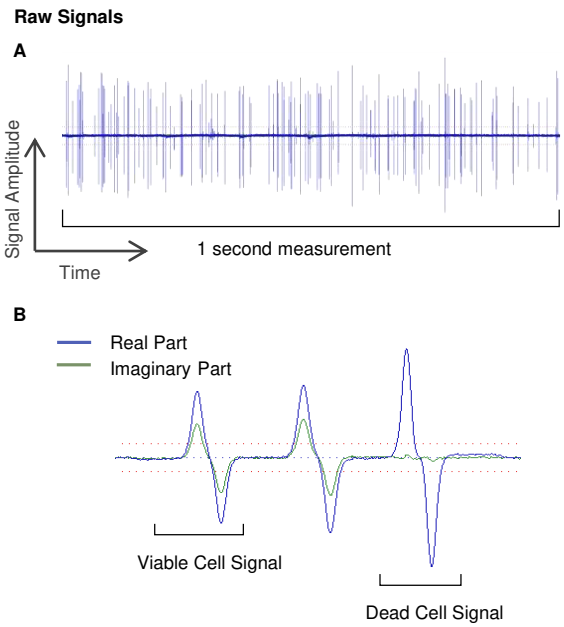

Figure 2: Impedance signals of BL2 cells.

A) Raw signal of 1 second of a measuremet. Each signal amplitude spike corresponds to the passage of a cell through electric field between the electrodes of the microfluidic chip.

B) Enlarged section of the raw signal, revealing the impedance response of two viable cells and one dead cell. The raw signal consists of a real part (blue line) and an imaginary part (green line). An algorithm is processing the raw signal and identifies the peaks of the real and imaginary part signals. From the peaks, impedance phase angle and amplitude are calculated for each cell.

Data analysis using AmphaSoft is straightforward. Several features, such as gating of populations, overlay of different plots and statistical information about gate contents are available. Templates can be used to facilitate repetitive measurement series and comprehensive reports summarize measurement settings and results.

Phase-Amplitude Scatterplots

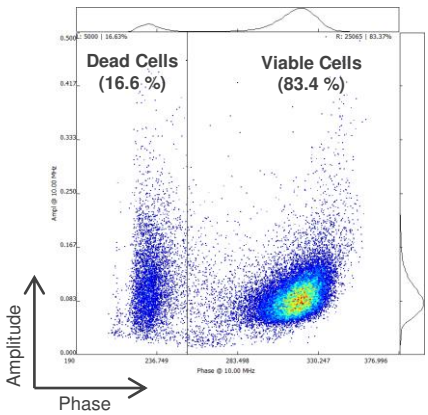

Figure 3: Phase – Amplitude scatterplot of a BL2 culture with 83.4 % viable cells, measured at a frequency of 10 MHz. These scatterplots are generated automatically by the AmphaSoft software. The top and right part of the plot display phase and amplitude histograms, respectively.
When cells are measured using multiple frequencies, the results of each frequency will be displayed in an individual plot, and the impedance response of a cell to various frequencies can be observed at a glance by having all plots side-by-side.
The left population in the scatterplot above corresponds to dead cells, while the right population corresponds to the viable population. Color-coding is used to visualize dense areas in the plot. For data analysis, a set of gates and gate statistics and reporting are available, among many other features.

IFC SAMPLE PREPARATION AND MEASUREMENT

Due to the label-free nature of the IFC measurement, sample preparation is minimal. It typically consists of a dilution with a measurement buffer and a filtration step.

- Take a sample from the cell culture

  Typically 50 – 100 µl

- Dilute the sample with measurement buffer

  Depending on the cell type and application, the recommended measurement buffer may vary. For BL2 cells, the Amphasys measurement buffer AF6 was used. Typically, dilutions between 1:1 to 1:10 are recommended, if samples are taken directly from culture.

- Filter to remove particle agglomerates

  This step depends on the size and purity of the cell culture and the particle to chip channel cross section ratio. For BL2 cells, a filtration step was not required.

The measurement process starts with the aspiration of the sample through the microfluidic chip and ends when a certain stop condition (time, number of cells, volume) is reached, or when the user stops the measurement manually.

For the measurement of BL2 cells, the following experimental setup was chosen:

- 50 µm x 50 µm measurement chip (C-Chip)
- Gain settings:
  - Modulation – 5
  - Amplification – 4
  - Demodulation – 0
- Flow rate: Approximately 40 µl/min
- Measurement frequencies: 2 MHz and 10 MHz
- Triggering source and component: Real part, positive
- Stop conditions: Depending on experiment, typically 60 seconds or 25'000 cells

CELL VIABILITY OF DIFFERENT CULTURES

The impedance signals of viable and dead BL2 cells show marked differences (Fig. 1 B). As a result, both appear as distinct clouds in Phase-Amplitude scatterplots. Dead cells form a confined cloud at low impedance phase angle, whereas viable cells typically form a broader distribution at a higher phase. For cells that were cultivated for a long time without supplying fresh medium, the phase angle of the viable population decreases, as the cells start to become apoptotic and die.

Figure 3 shows Phase-Amplitude scatterplots of different BL2 cell cultures, revealing large differences in cell viability between fresh and old cell cultures. Viable and dead cell clusters can easily be separated using a vertical line gate.

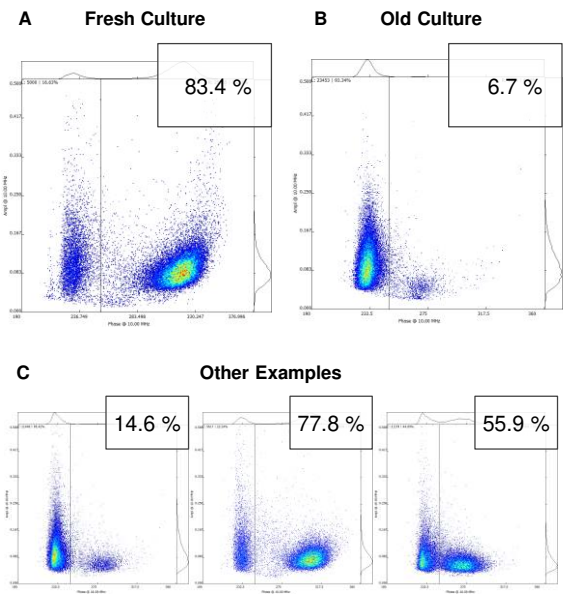

Figure 3: Viability analysis of different BL2 cell cultures. The inserted boxes indicate the percentage of viable cells. Viable cells were quantified by a vertical line gate. All plots were acquired at a frequency of 10 MHz.

A) Scatterplot of a fresh cell culture, showing a high viability of 83.4 %.
B) Scatterplot of an old culture, where no more fresh medium was supplied.
C) Examples of other cultures at different cultivation timepoints.

In Figure 4, the cell viability of a dense culture was monitored over 6 days. A clear phase shift of the viable population, as well as a steep decrease in cell viability can be observed after day 1.

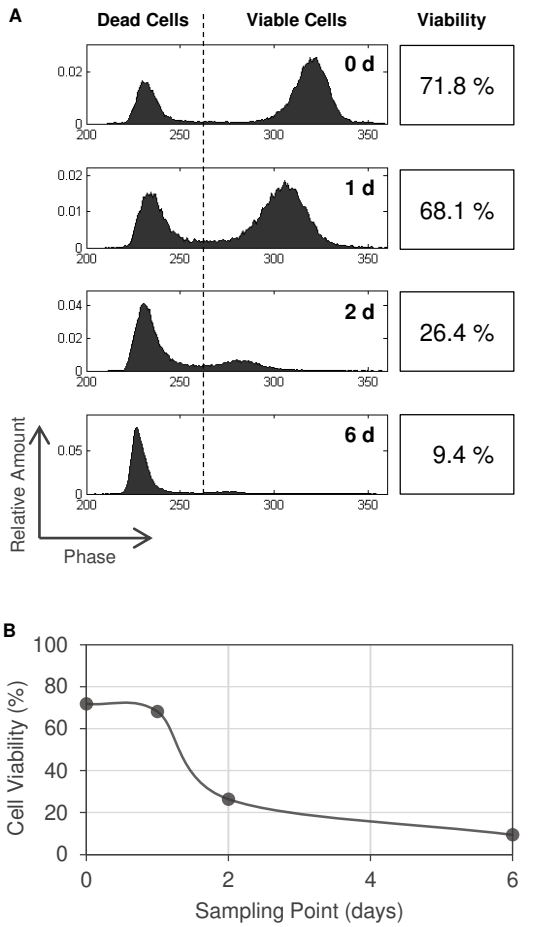

Figure 4: Following BL2 culture progression at a measurement frequency of 10 MHz.

A) Impedance phase histograms and the corresponding cell viability at 0, 1, 2 and 6 days. The dashed vertical line separates viable from dead cells. Besides a decreasing viability, also a phase shift of the viable population can be observed.

B) Cell viability over the measurement period of 6 days.

CELL CONCENTRATION

In addition to cell viability, the Ampha Z32 measurements also provide accurate concentrations of total, viable and dead cells. In order to determine the linear measurement range for the concentration, a dilution series was prepared.

- A BL2 cell sample was harvested by centrifuging the cells for 2 min at 1'500 rpm, followed by discarding the supernatant and resuspending the cells with fresh measurement buffer.
- Afterwards, a dilution series with 1:3, 1:9 and 1:27 dilutions was prepared and measured in triplicate.
- The 1:3 dilution was quantified with a Neubauer counting chamber.

The results of the dilution series are shown in Fig. 5 A. The concentrations show a linear behavior. However, high cell concentrations (> 2 × 10^6 cells / ml) lead to signal overlaps due to very close or coincident passage of multiple particles (Fig. 5 C). This leads to an underestimation of the cell concentration. In practice this is not an issue, as the IFC measurements involve a dilution step with the measurement buffer. The dilution factor can be adjusted accordingly to make the sample concentration fall into the linear range.

The results in Fig. 5 B indicate a reproducible viability determination, independent of the cell concentration.

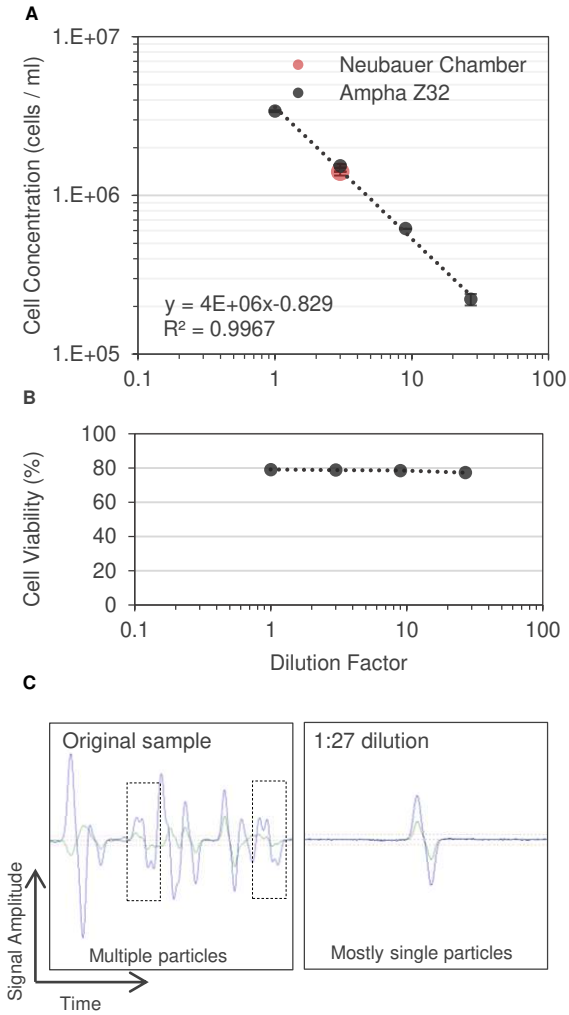

Figure 5: Dilution series to investigate the linear range of the concentration determination.

A) Cell concentrations of 4 different dilutions, each measured in triplicate. The average standard deviation is 3 %.

B) Cell viabilities of different sample dilutions. The viability determination is not affected by the cell concentration (two-tailed unpaired t-test, $p > 0.05$)

C) Examples of the signal properties of the original sample and the 1:27 dilution, both measured at a frequency of 2 MHz. The highly concentrated original sample shows signal disturbances (leftmost signal) and doublet signals (dashed boxes), originating from very close or coincident passage of cells through the detection volume. By contrast, the 1:27 diluted sample shows almost exclusively well-defined and discrete particle signals.

INDUCTION OF APOPTOSIS

Staurosporine is a protein kinase inhibitor and apoptosis inducer [3]. The response of BL2 cells to staurosporine treatment was investigated with a time course experiment. Fig. 6 shows a distinct impedance change of the viable cell population (phase shift), followed by continuous cell death.

This data, together with the observation that viable cells in old cultures appear at lower phase angles (Fig 4 A), suggests that apoptotic cells undergo a phase shift from a high phase angle (≈ 325°, viable and proliferative cells) via an intermediate transition state (apoptosis) to a low phase angle (≈ 235°, dead cells). Therefore, the phase angle could be an interesting indicator of the cell state.

CONCLUSIONS

We report the characterization of a cancer cell line by impedance flow cytometry, a powerful label-free method for the measurement of electric properties of cells at the single-cell level. After dilution of a cell culture sample with a measurement buffer, cells can be acquired immediately at a rate of > 1'000 cells/s.

During and after the measurement, impedance data is displayed in Phase-Amplitude plots. Sophisticated data analysis tools allow to rapidly identify and quantify viable and dead cells and to determine the respective cell concentrations.

We observed that the impedance phase provides information about the cell state, i.e. if a cell is viable, apoptotic or dead.

Thus, IFC is a promising technology for cell characterization in a label-free and rapid manner.

Potential applications include

- Monitoring concentration and viability of cell cultures
- Dose-response or time-course of apoptosis-inducing, cytotoxic or other agents

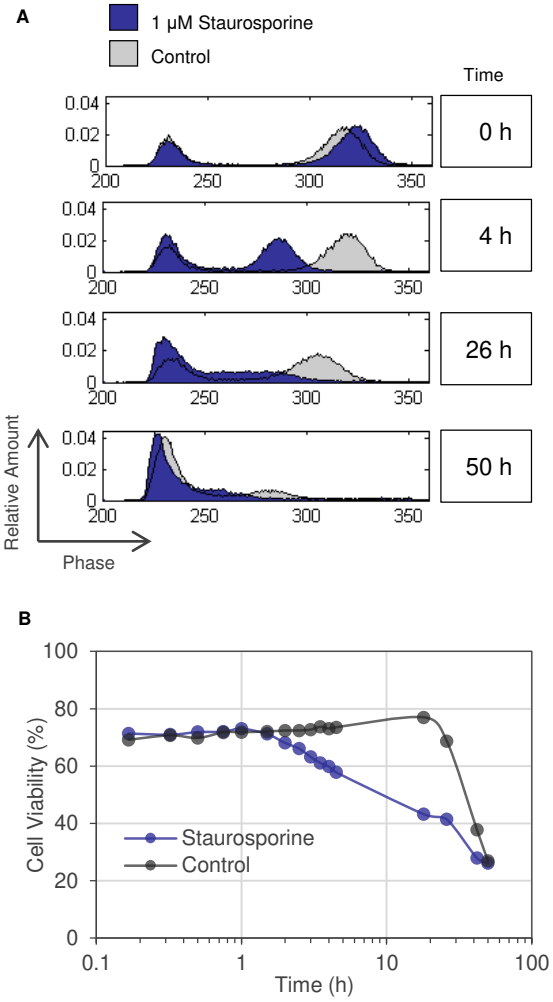

Figure 6: Induction of apoptosis in BL2 cells by staurosporine treatment. Cultures were treated with either 1 µM staurosporine in DMSO or just DMSO as a control.

A) Phase histograms of BL2 cells at various timepoints after treatment with 1 µM staurosporine. The viable cell population showed a rapid initial phase shift (t < 10 min), followed by a stable phase, until the cells started to die (t > 1.5 h). Phase histograms were calculated based on impedance data at 10 MHz.

B) 50 hours viability time course after staurosporine treatment of BL2 cells. After 1.5 h, the viability of the treated culture rapidly decreased, while the control culture was still in a growth state. After 18 h, the viability of the control culture also decreased.

REFERENCES

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3. Belmokhtar CA, Hillion J and Ségal-Bendirdjian E (2001), Staurosporine induces apoptosis through both caspase-dependent and caspase-independent mechanisms. *Oncogene*, 20 (26): 3354 – 3362

Contact us now for more information

Amphasys AG
Technopark Lucerne
CH-6039 Root D4
Switzerland

info@amphasys.com
+41 41 541 91 20
www.amphasys.com