Characterization of Apoptosis & Viability in Cancer Cells by Impedance Flow Cytometry

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Introduction

Impedance Flow Cytometry (IFC) is a technique to rapidly characterize large amount of cells at the single-cell level. The readout depends solely on the electrical properties of the cells, thus no labelling is required and sample preparation is minimal.

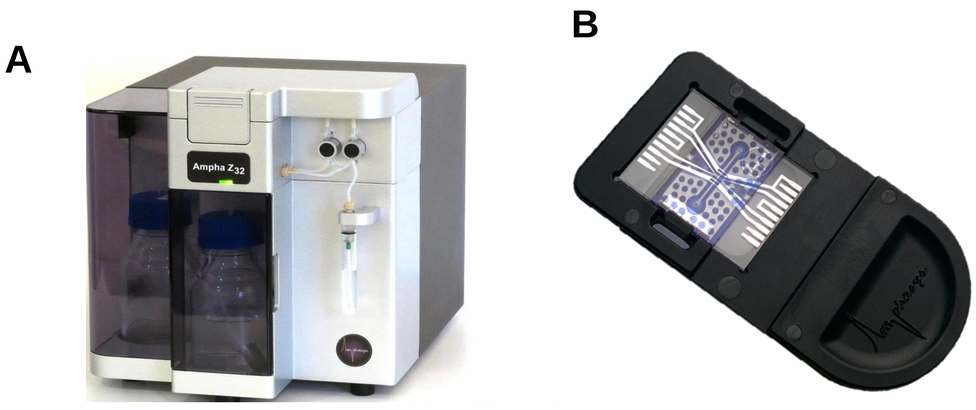

Figure 1: *A) Amphasys Ampha Z32 Impedance Flow Cytometer*
B) Amphasys Microfluidic Chip

IFC delivers highly repeatable results due to the high number of cells measured (10'000's of cells per minute) and is exceptionally well suited for applications that require a quick time to result:

- High resolution viability determination
- Concentration determination of viable, apoptotic & dead cells

Here we report the characterization of BL2 (human cell line, originally isolated from a Burkitt lymphoma) cell cultures under various conditions.

Fast, Label-Free Cell Viability

Cells in suspension are pumped through the microchip, where they are exposed to an electrical field with alternating current. Viable and dead cells have different electric properties and influence the impedance signal differently – allowing us to clearly distinguish between viable and dead cells.

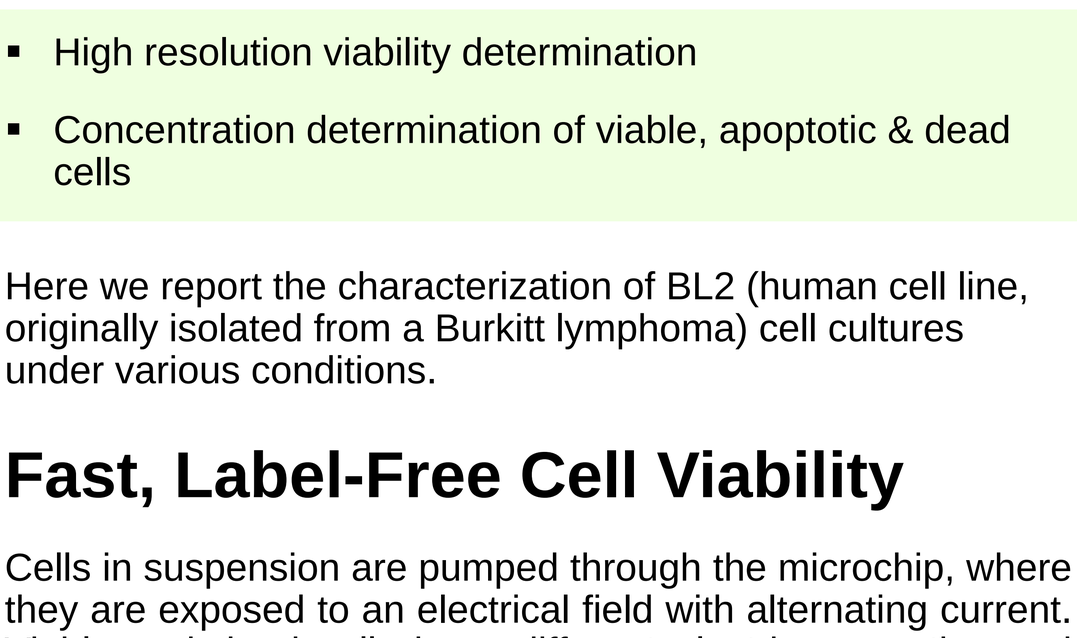

Figure 2: *A) Phase-amplitude scatterplot of a fresh BL2 culture with 83.4% viable cells. B) Impedance phase histograms and the corresponding cell viability after 0, 1, 2 and 6 days.*

Monitoring of Viability & Apoptosis

Staurosporine is a protein kinase inhibitor and apoptosis inducer[1]. The response of BL2 cells to Staurosporine treatment was investigated with a time course experiment. Figure 3A reviews a distinct impedance change of the viable cell population upon Staurosporine treatment, followed by continuous cell death.

This data, together with the observation that viable cells in old cultures appear at lower phase angles (Fig. 2B) shows that apoptotic cells undergo a phase shift from a high phase angle (~325°, proliferating cells), via an intermediate transition state (apoptosis, highlighted in green) to a low phase angle (~235°, dead cells).

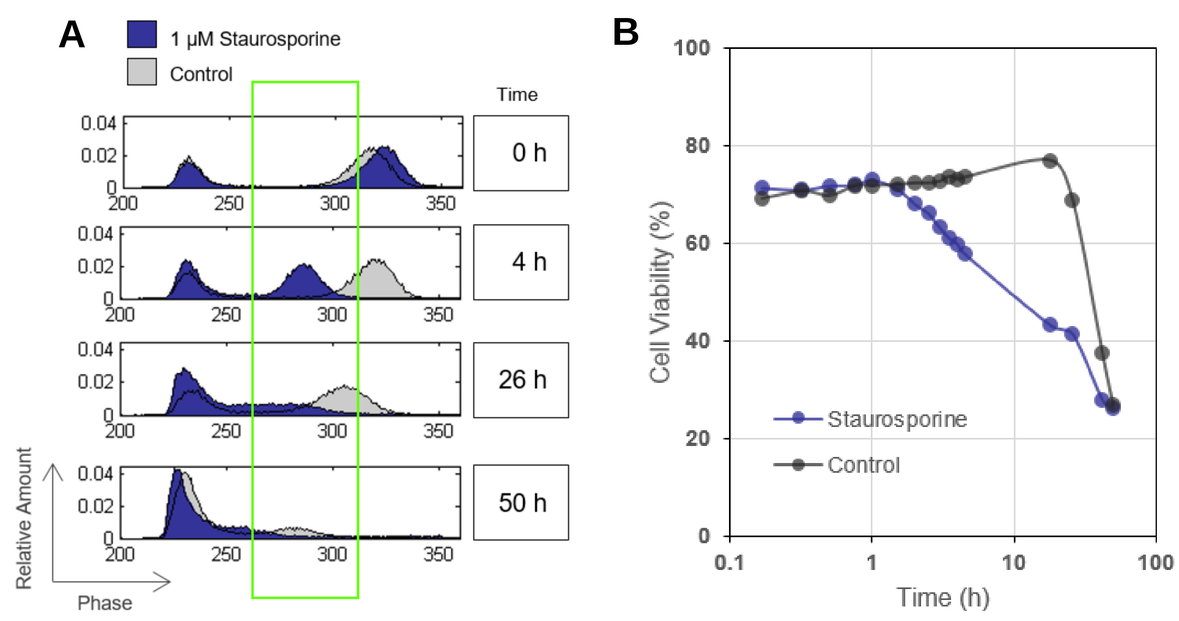

Figure 3: *A) Histogram of BL2 cells at various time points after treatment with 1µM Staurosporine. B) 50 hours viability time course after Staurosporine treatment.*

Conclusions

We report the characterization of a cancer cell line by impedance flow cytometry, a powerful label-free method for measurement of the electrical properties of single cells. The impedance (electrical resistance in alternating current) data is displayed in phase-amplitude plots and data analysis tools allow to rapidly identify and quantify viable and dead cells and to determine the respective cell concentrations.

Applications in bioprocess optimization and drug development include:

- Monitoring health of cell cultures
- Dose-response or time-course experiments of cytotoxic or apoptosis inducing agents

Acknowledgement
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Reference
1. Belmokhtar CA, Hillion J and Segal-Bendirdjian E (2001), Oncogene, 20 (26): 3354 - 3362