



ÓBUDAI EGYETEM
ÓBUDA UNIVERSITY

PhD THESIS BOOKLET

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**INTEGRATED MICROFLUIDIC SYSTEMS
FOR CELL ANALYSIS**

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1 Background to the research

Microfluidic systems have emerged as a highly promising research field with applications in biology, chemistry, medical diagnostics, and pharmaceutical sciences¹. One of their most important roles is the implementation of sample preparation processes in Lab-on-a-Chip (LoC) systems. These devices integrate laboratory preparation and analytical functions within a single platform, enabling complex sensing and diagnostic tasks to be performed in a miniaturized format. Owing to their versatility and broad applicability, microfluidic technologies have attracted significant research interest over the past decade².

As LoC technologies have advanced toward cellular and tissue-level applications, microphysiological systems and Organ-on-Chip (OoC) platforms have emerged as powerful tools for the investigation of cellular behaviour and tissue function. By recreating physiological microenvironments and enabling interactions between different cell types and tissues, these systems facilitate the study of complex biological processes and responses to external stimuli³. OoC devices have considerable potential in drug discovery and development, as active compounds can be delivered under precisely controlled conditions while their effects are monitored in real time within biologically relevant models. Furthermore, these platforms offer an attractive alternative to animal experimentation by utilizing human-derived cells and tissues in controlled artificial environments. Since metabolic and physiological responses often differ substantially between animal models and humans, OoC systems may provide more predictive results during preclinical testing.

Beyond pharmaceutical applications, OoC technologies contribute significantly to cancer research by enabling the investigation of tumour tissues, tumour progression mechanisms⁴, responses to therapeutic agents⁵, and interactions between malignant and healthy cells⁶. They have also been employed for the isolation and characterization of cancer cells⁷. Consequently, OoC platforms are expected to play an increasingly important role in the advancement of personalized medicine.

The aim of this research was the design, fabrication, and characterization of microfluidic structures capable of generating controlled chemical microenvironments, trapping cells, and monitoring their properties. In addition to supporting cell-based analytical studies, the integration of microelectrodes enables the application of non-invasive, label-free measurement techniques, allowing cellular properties to be investigated without perturbing the biological system. The generation of microscale chemical gradients facilitates the controlled delivery of active compounds at defined concentrations, while the transparent device architecture permits optical observation of trapped cells. Combined with fluorescence-based measurement methods, the platform enables detailed investigation of cellular responses to various substances. Monitoring cell viability and proliferation represents an essential step toward the development of a versatile analytical tool that may contribute to cancer research and provide new opportunities for rapid bacterial antibiotic susceptibility testing.

The fabrication of the microfluidic chips and integrated electrodes, as well as the subsequent experimental investigations, were carried out at the Microsystems Laboratory of the Institute for Technical Physics and Materials Science of the HUN-REN Centre for Energy Research.

2 Objectives

The primary objective of my research was to develop microfluidic systems that apply electrical measurement techniques instead of optical methods. This approach was motivated by the fact that fluorescent dyes commonly used in optical assays may influence cellular function, alter cellular behaviour, and exhibit cytotoxic effects. Impedance spectroscopy is a suitable method for monitoring cellular responses without external interference and serves as the basis of numerous chemical and cell-analytical techniques. Therefore, the aim of this work was to create a microfluidic device suitable for impedance spectroscopy, enabling non-invasive and label-free measurements within the microfluidic structure.

In studies involving cell cultures, monitoring the distribution and concentration of therapeutic agents is of critical importance. The initial phase of my work focused on investigating and monitoring the behavior of substances introduced into microfluidic structures. To achieve this, I studied the controlled generation of chemical gradients in a microfluidic system specifically designed for this purpose.

The next stage of the development was dedicated to the implementation of cell trapping in microfluidic environments, which is essential for the localized investigation of cellular properties. For this reason, various cell-trapping approaches were considered in appropriately modified microchannel geometries.

Developing methods for monitoring cell viability was an integral part of this research, as the assessment of cellular viability is crucial in investigating the mechanisms of therapeutic compounds. For the simultaneous optical and electrical characterization of cell viability, a printed circuit board incorporating an integrated microfluidic structure was developed, enabling both fluorescence- and impedance- based measurements. During the development, electrodes were integrated into the system and their functionality was experimentally validated.

To demonstrate the feasibility of the developed platform (proof-of-concept), yeast cells (*S. cerevisiae*) were used as model organisms throughout the study, as they are considerably easier and safer to handle than mammalian or human cells. The collaboration and support of the Drug Resistance Research Group at the HUN-REN Research Centre for Natural Sciences were indispensable for carrying out the cell-based experiments.

3 Test methods

3.1 Microfluidic system design

The microfluidic structures were fabricated using soft lithography and photolithography techniques. The geometrical layouts were designed using CleWin5 and CleWin6 (WieWeb software). The chromium mask of the pattern was produced by direct lithography (Heidelberg DWL 66). A mask aligner (Süss MicroTech MA6) was used to transfer the lithographic pattern onto silicon or glass substrates to achieve the desired microstructures. The negative mold of the structures was formed by exposing SU-8, a negative-tone photoresist. For soft lithography, a two-component polydimethylsiloxane (PDMS) polymer (Sylgard 184) was used.

During electrode integration, thin metal layers were deposited using vacuum-based techniques, including sputtering (Vaksis MiDAS 3M) and thermal evaporation (AJA International ATC-M). The electrode patterns were defined using lift-off lithography. The materials of the electrodes were analyzed by scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) (Zeiss LEO 1540 XB), while further structuring was carried out using focused ion beam (FIB) milling (ThermoScientific Scios 2).

3.2 Fluorescence-based measurements

The formation of the chemical gradient and the optical analysis of the trapped cells were performed using fluorescence spectroscopy. Measurements were carried out with a Zeiss Axio Vert.A1 fluorescence microscope equipped with a Zeiss Axio 512 mono camera. The images acquired at specific wavelengths were processed using ZEN 2.6 (Blue Edition) software, and diffusion profiles were extracted using ImageJ 1.52a.

3.3 Electrical measurements

For the functional validation of the electrodes and the analysis of trapped cells, electrochemical impedance spectroscopy (EIS) was applied. Measurements were conducted using a PalmSense4 potentiostat, and the data were evaluated with the PS Trace 5.8 software.

4 New scientific achievements

[T1] I designed and developed a microfluidic system for the analysis of free molecular diffusion, that enables the determination of diffusion coefficients of fluorescent molecules based on optical detection through the monitoring and analysis of time-dependent concentration gradient profiles.

[P1] [C2] [C3]

- (i) The structure of the microfluidic system uniquely enables the analysis of free molecular diffusion without the use of obstacles or membranes. I demonstrated that the designed architecture – by combining with an appropriate measurement protocol – ensures the establishment of a constant surface concentration in the main channel, allowing the application of the complementary error function for the mathematical description of the diffusion process. The symmetrical channel layout, the large (7 mm) diameter inlet reservoirs, and the sample injection capillaries with high hydrodynamic resistance – having the dimensions of $10\text{ }\mu\text{m}$ (depth) $\times$ $6\text{ }\mu\text{m}$ (width) $\times$ $800\text{ }\mu\text{m}$ (length) – minimize convective effects arising from hydrostatic pressure differences.
- (ii) I demonstrated that the developed device and method are capable of reliably eliminating the effects of processes generating local optical noise (e.g., protein aggregation) and enable the rapid determination of protein diffusion coefficients within 10 minutes.
- (iii) Using a Python-based data analysis code developed for the evaluation of optical measurements, I determined the diffusion coefficients of three test molecules of different size scales – BSA, IgG, and RhB. Applying a defined 1% function-fitting error criterion, the obtained values showed adequate agreement with literature data, with deviations in the range of 1–10%, depending on the molecular size and the reference measurement technique.

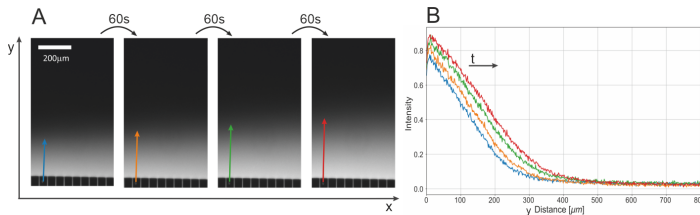


Figure 1: (A) Fluorescent detection of BSA diffusion with 1 min differences and (B) the respective vertical intensity profiles

[T2] I developed a PDMS-based microfluidic system incorporating integrated electrodes and a vertical trapping structure, capable of immobilizing individual cells or groups of cells and enabling the parallel optical and electrical monitoring of cell numbers. [P2] [P3] [C4]

- (i) I demonstrated that the filling of the individual channels of the microfluidic system with culture medium can be monitored by continuous impedance measurements performed at a frequency of 1 kHz.
- (ii) Using electrochemical impedance spectroscopy, I characterized the effect of cell number on the impedance spectrum in case of *S. cerevisiae* yeast cells in the developed microfluidic system. I determined that, when fitting the proposed modified Randles equivalent circuit model, the charge-transfer resistance correlated strongly with changes in cell number, yielding a correlation coefficient of $r = 0.92$ for low cell numbers (< 20 cells).

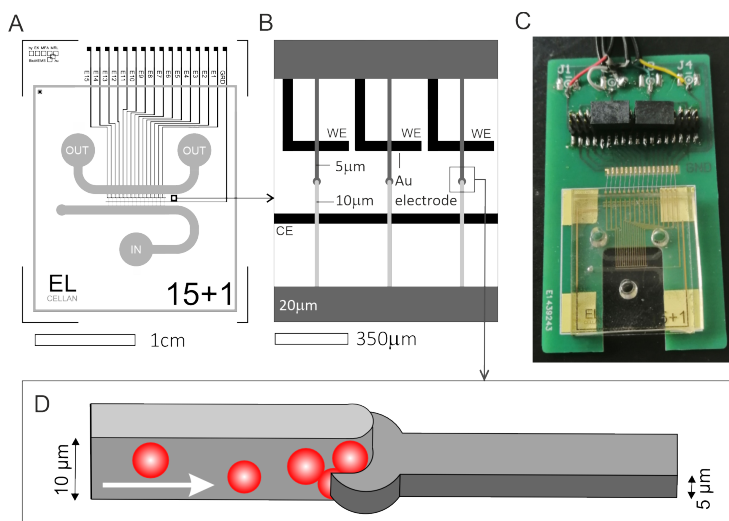


Figure 2: (A) Layout of the microfluidic chip (light grey) and the underlying electrode system (black). (B) Closeup on the trapping cross-channels and the electrodes. (C) The designed and manufactured PCB containing a window for optical screening and the assembled microfluidic device. (D) Schematic figure of the trapping site showing how cells are caught in the vertically narrowing channel.

[T3] I developed a microfluidic system with channels and trapping structures fabricated directly in SU-8, together with integrated electrodes, capable of the parallel optical and electrical monitoring of cell viability in captured cell populations.

[C5] [C9]

- (i) By optimizing the material, geometry, and arrangement of the integrated electrodes, I improved the sensitivity of the impedance-based analytical platform. I demonstrated that the applied platinum electrodes enhanced the long-term stability of the system, while increasing the electrode surface area and decreasing the inter-electrode distance improved the charge-transfer resistance characteristics.
- (ii) Using electrochemical impedance spectroscopy, I analysed the UV irradiation induced changes of the cell viability in *S. cerevisiae* suspensions containing 10^6 cells/ml. I demonstrated that the developed system is applicable for electrically monitoring cell viability through changes in the parameters of the fitted equivalent circuit model. I determined that a decrease in yeast cell viability resulted in a significant increase in charge-transfer resistance and a decrease in the constant phase element associated with double-layer capacitance.
- (iii) By experiments involving human MES-SA cancer cells, I demonstrated that, following appropriate geometrical adaptation, the microfluidic device was capable of monitoring cell death through the combined application of optical and electrical measurement techniques.

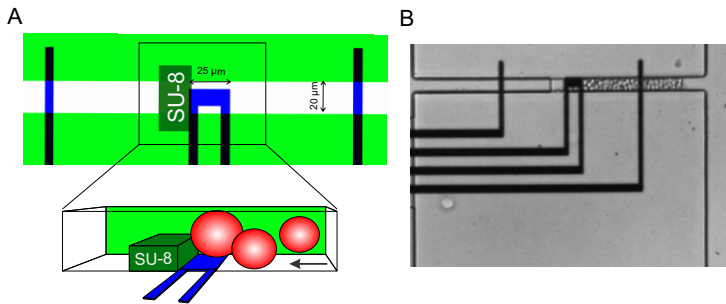


Figure 3: (A) Schematic illustration of the cell-trapping structure showing the SU-8 microchannel (green), a magnified view of the trapping region (center), the electrode arrangement (blue), the central barrier (dark green), and the cells (red). (B) Microscopic image of trapped yeast cells.

5 Potential use of the results

The device I developed enables non-invasive, real-time investigation of various cell types, thus opening new perspectives for the development of compact microfluidic systems integrated with electrical measurement techniques. This approach may contribute to significant advances in several fields, including the study of disease mechanisms, the investigation of therapeutic strategies using Organ-on-Chip systems, and the accelerated analysis of antibiotic susceptibility in microfluidic environments.

The proposed microfluidic and Organ-on-Chip platforms represent experimental systems that allow the long-term maintenance of living cells or tissues under controlled laboratory conditions, while enabling continuous monitoring through integrated sensing technologies. These emerging platforms are expected to enhance our understanding of human biology and to accelerate the development and testing of new pharmaceutical compounds.

The results presented in this PhD thesis have been directly utilized in the INBIOM (TKP2021-EGA-04) project. The primary objective of this project is to demonstrate compact, modular measurement principles that enable direct analysis of cell populations, including applications in drug testing. The detection of cells, as well as the monitoring of their behavior and viability, can be further simplified through the use of integrated sensing systems.

The optical and electrical measurement approaches developed in this work (EIS, EIT) may support therapeutic design processes, including drug resistance studies, and may also contribute to the development of personalized treatment strategies.

6 Scientific publications related to the thesis points

6.1 Publications

- [P1] Lilia Bató, Péter Fürjes, "Diffusion coefficient measurement with fluorescent detection in free-diffusion based microfluidics" *Microfluidics and Nanofluidics*, 28 (8) pp. 56, 2024; doi:10.1007/s10404-024-02752-w
- [P2] Lilia Bató, Péter Fürjes, "Vertical Microfluidic Trapping System for Capturing and Simultaneous Electrochemical Detection of Cells" *Sensors*, 24 (20), pp. 6638, 2024; doi: 10.3390/s24206638
- [P3] Lilia Bató, Péter Fürjes, "Microfluidic System with Integrated Electrode Array for High-Throughput Electrochemical Impedance Spectroscopy Analysis of Localised Cells" *Proceedings*, 97 (1), 187, 2024; doi: 10.3390/proceedings2024097187
- [P4] Lilia Bató, János M. Bozorádi, Zoltán Vizvári and Péter Fürjes, "Microfluidic System With Integrated Electrode Array for Impedance Tomography of Cell Cultures," 2025 Symposium on Design, Test, Integration and Packaging of MEMS/MOEMS (DTIP), Split, Croatia, 2025, pp. 1-5; doi: 10.1109/DTIP66728.2025.11099198
- [P5] Lilia Bató, Péter Fürjes, János M. Bozorádi, Vladimir Tadic, Péter Odry, Zoltán Vizvári, "Sensitivity Analysis of Localized Electrochemical Impedance Spectroscopy Towards Tomography-on-a-Chip" *Sensors*, 25 (20), pp.6393, 2025, doi:10.3390/s25206393

6.2 Conference Presentations

- [C1] Lilia Bató and Péter Fürjes, "Individual cell trapping and viability testing in microfluidic device," *Matrafüred – International Meeting on Chemical Sensors*, June 12-17, 2022, Visegrád, Hungary (poster)
- [C2] Lilia Bató and Péter Fürjes, "A fluorescent detection method to measure the diffusion coefficients of proteins in a free-diffusion based microfluidic system," *Lab-on-a-Chip and Microfluidics Europe*, June 21-22, 2022, Rotterdam, The Netherlands (poster)
- [C3] Lilia Bató and Péter Fürjes, "An obstacle-free microfluidic system for monitoring protein diffusion," *MBFT XXIX. Congress*, August 28-31, 2023, Budapest, Hungary (lecture)

- [C4] Lilia Bató and Péter Fürjes, "Microfluidic system with integrated electrode array for EIS analysis of localised cells," Eurosensors 2023 Conference, September 10 - 13, 2023, Lecce, Italy (poster)
- [C5] Lilia Bató, János M. Bozorádi, Zoltán Vizvári, Péter Fürjes "Microfluidic device for impedance tomography" Mátrafüred - International Conference on Chemical Sensors, June 16-21, 2024, Visegrád, Hungary (poster)
- [C6] Lilia Bató, János M. Bozorádi, Zoltán Vizvári, Péter Fürjes, "Microfluidic device for EIS and optical monitoring of cells" EUROoCS Annual Meeting, July 3-5, 2024, Milan, Italy (poster)
- [C7] Lilia Bató, János M. Bozorádi, Zoltán Vizvári, Péter Fürjes, "Microfluidic device with integrated microelectrodes for enhanced EIS sensitivity ," Eurosensors 2024 Conference, September 1 - 4, 2024, Debrecen, Hungary (lecture)
- [C8] Lilia Bató, János M. Bozorádi, Zoltán Vizvári, Péter Fürjes, "Microfluidic System With Integrated Electrode Array for Impedance Tomography of Cell Cultures," DTIP 2025 Conference, June 1 - 4, 2025, Split, Croatia (lecture)
- [C9] Lilia Bató, János M. Bozorádi, Zoltán Vizvári, Péter Fürjes, "Real-Time Cell Viability Testing in Microfluidic System by EIS," MPS 2025 World Summit, June 10 - 13, 2025, Brussels, Belgium (poster)

7 Additional scientific publications

- [P6] Bányai Anita, Bató Lilia, Leelőssyné Tóth Eszter, Varga Máté, Fürjes Péter, "Áramlástan jelenségek mikroszkopikus mérettartományban – mikrofluidikai rendszerek és alkalmazásaik", Fizikai Szemle (0015-3257): 73 (11) pp. 383–389, 2023
- [P7] Barbara Beiler, Ágnes Sáfrány, Lilia Bató, Zsombor Szomor, Miklós Veres "Effect of binary porogen mixtures on polymer monoliths prepared by gamma-radiation initiated polymerization" Heliyon, 10 (19), 2024; doi: 10.1016/j.heliyon.2024.e38852
- [P8] Zsombor Szomor, Lilia Bató, Silvia Stagl, Orsolya Hakkel, Attila Sulyok, Csaba Dücső, Zsófia Baji and Péter Fürjes, "Non-stoichiometric titanium-oxide gate electrodes for EGFET based pH sensors," Proceedings, 97 (1), 187, 2024
doi: 10.3390/proceedings2024097187
- [P9] Zsombor Szomor, Lilia Bató, Orsolya Hakkel, Csaba Dücső, Zsófia Baji, Attila Sulyok, Erzsébet Dodony, Katalin Balácsi, János M. Bozorádi, Zoltán Szabó and Péter Fürjes, "Characterisation of Titanium-Oxide Thin Films for Efficient pH Sensing in Low-Power Electrochemical Systems," Sensors, 25 (19), 6113, 2025
doi: 10.3390/s25196113

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- [2] J. P. Lafleur, A. Jönsson, S. Senkbeil, and J. P. Kutter, "Recent advances in lab-on-a-chip for biosensing applications," *Biosensors and Bioelectronics*, vol. 76, pp. 213–233, 2016.
- [3] C. Zhang, Z. Zhao, N. A. A. Rahim, D. van Noort, and H. Yu, "Towards a human-on-chip: Culturing multiple cell types on a chip with compartmentalized microenvironments," *Lab on a Chip*, vol. 9, no. 22, 2009.
- [4] D. Wood, D. M. Weingeist, S. Bhatia, and B. Engelward, "Single cell trapping and DNA damage analysis using microwell arrays," *National Academy of Sciences*, vol. 107, no. 22, pp. 10008–10013, 2010.
- [5] D. Caballero, S. Kaushik, V. M. Correlli, J. M. Oliveira, R. Reis, and S. C. Kunku, "Organ-on-chip models of cancer metastasis for future personalized medicine: from chip to the patient," *Biomaterials*, vol. 149, pp. 98–115, 2017.
- [6] E. Biselli, E. Agliari, A. Barra, F. R. Bertani, A. Gerardina, A. De Ninno, A. Mencatini, D. Di Giuseppe, M. Fabrizio, G. Schiavoni, V. Lucarini, E. Vacchelli, G. Kroemer, C. Di Natale, E. Martinelli, and L. Buscinaro, "Organs on chip approach: a tool to evaluate cancer-immune cells interactions," *Scientific Reports*, vol. 7, no. 1, 2017.
- [7] K.-A. Hyun, T. Y. Lee, and H.-I. Jung, "Negative enrichment of circulating tumor cells using a geometrically activated surface interaction chip," *Analytical Chemistry*, vol. 85, no. 9, pp. 4439–4445, 2013.