Digital Tools for Automated Cancer Cell Identification and Cell Cluster Tracking using Adhesion Noise Spectroscopy

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INTRODUCTION

CMOS-based microelectrode arrays (CMOS MEAs) are used in biotechnological applications to record neural activity with high spatial (~15 µm) and high temporal resolution (~20 kHz bandwidth) using thousands of densely packed sensor sites [1]. A new application of CMOS MEAs is the **label-free and noninvasive detection of adherent cells** by studying the voltage noise from the resistive adhesion cleft [2-3]. The voltage noise is described as **cell adhesion noise** (CAN) and analyzed in terms of **spectral power density** (S_V) [4].

Here, we aim to assess cell proliferation, morphology, and motility of **colorectal cancer** (CRC) cells in a 2D culture. Therefore, we designed a machine-learning (ML) tool to distinguish between non-cancerous fibroblasts and CRC cells. Next, we correlated the adhesive properties of cancer cells on the CMOS MEA and their morphological and motility features by tracking cells over 72 h of cultivation time. The CAN-based cell detection is related to brightfield microscopy images. The extracted features offer a potent tool to infer kinetic and morphological information about cancer cells.

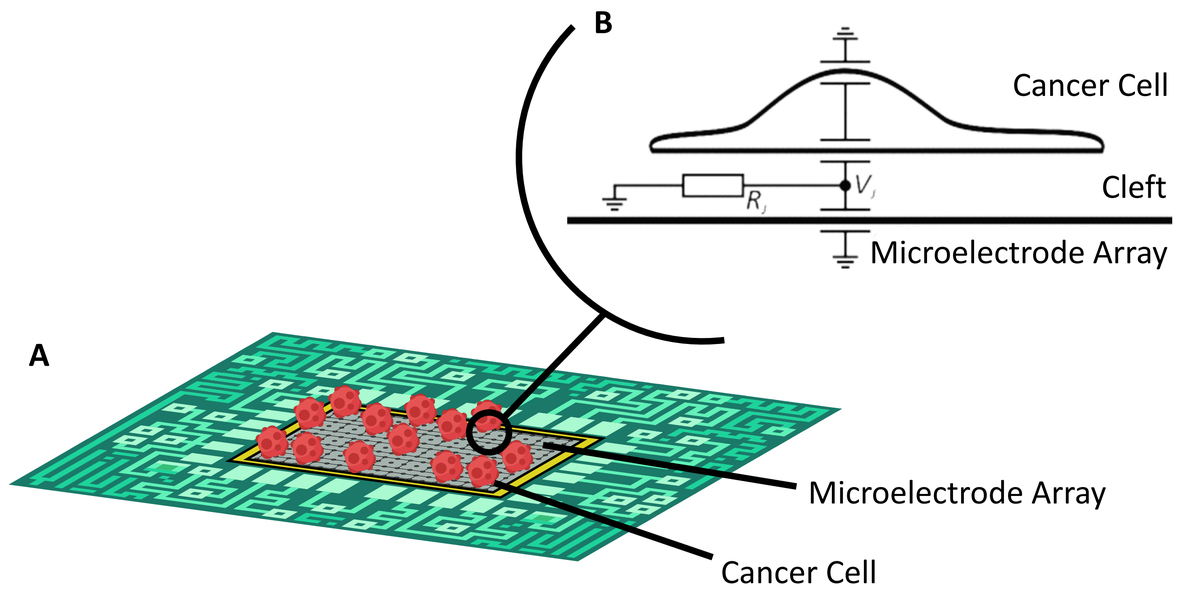

Figure 1: **Cancer cell–CMOS microelectrode array (CMOS MEA) interface**.
(A) Schematic illustration of cancer cells on CMOS MEA.
(B) Equivalent electronic circuit of the cell-CMOS MEA interface. R_J contributes significantly to the recorded extracellular voltage V_J regarding cell adhesion noise (CAN) [2].

METHODS

- CMOS MEA: **98304** hexagonal **recording** sites
  sensor size: **5.6 µm** x **6.5 µm**, sensitive area: 1.6 mm x 2.5 mm
- HT-29 colorectal cancer cell line
  Human dermal fibroblasts
- **Unsupervised** cell detection and tracking
- **Machine-learning**-based cell-type identification
- Cell proliferation, morphology, and motility

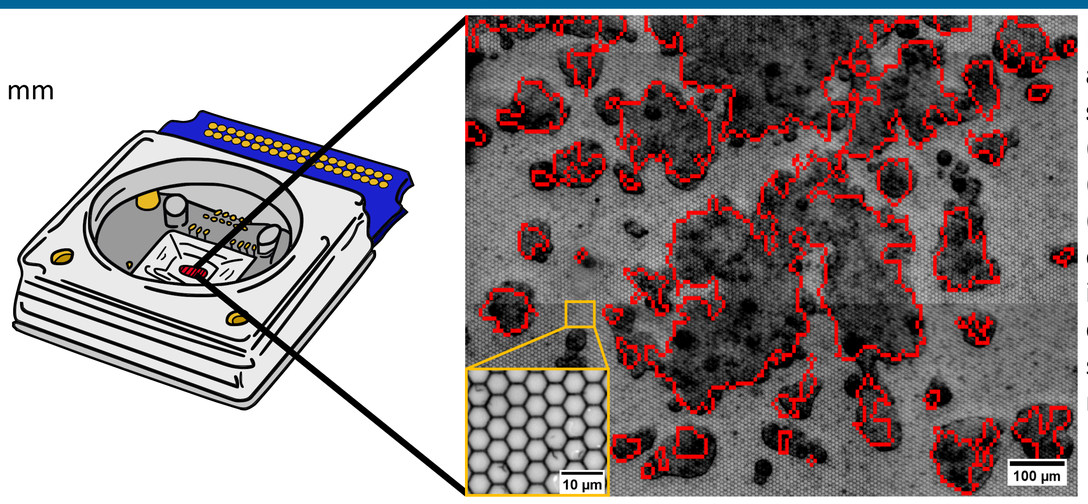

Figure 2: **Non-invasive detection of cell attachment using cell adhesion noise spectroscopy (S_V assessed at 300 kHz).** (left) Schematic CMOS microelectrode array (MEA) with high-density recording sites.
(right) Overlay of electrically identified HT-29 cells (red contours) with brightfield microscopic image of the CMOS MEA with adherent HT-29 cells (dark grey/black: cell clusters, light grey: sensor sites in the background). Inset: 50x magnification of sensor sites on the MEA. [5]

RESULTS

Machine Learning-based cell identification:

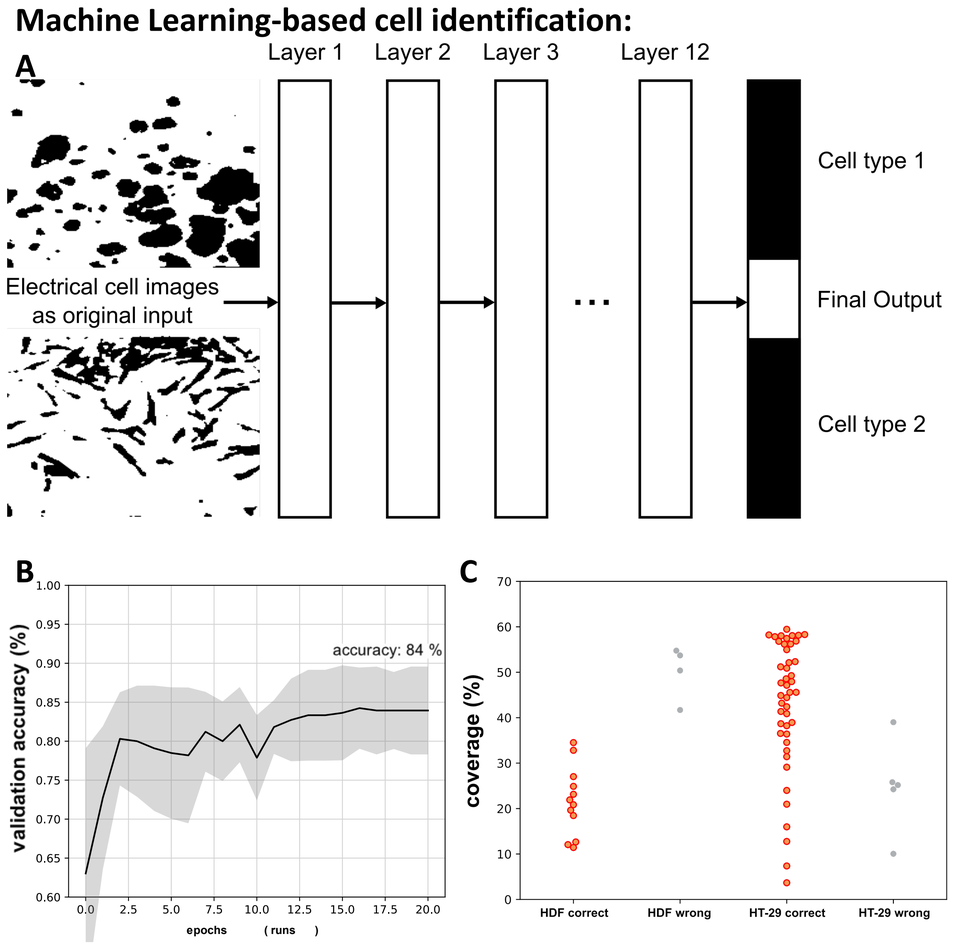

Figure 3: **Deep neural network for cell type classification.**
(A) The deep neural network processes the electrical images with a 12-layer machine learning model through successive filters, which come out increasingly purified to enable cell type classification. (B) Mean validation accuracy (black trace) with standard deviation (grey trace) of five cross-validated models on cell type classification shows 84 % correctly predicted cell types after 20 epochs (runs). (C) Correlation between cell-covered area and cell type-dependent right (orange dots with red contours) or wrong (grey dots) predictions shows no bias in cell type identification. [6]

Cell Tracking:

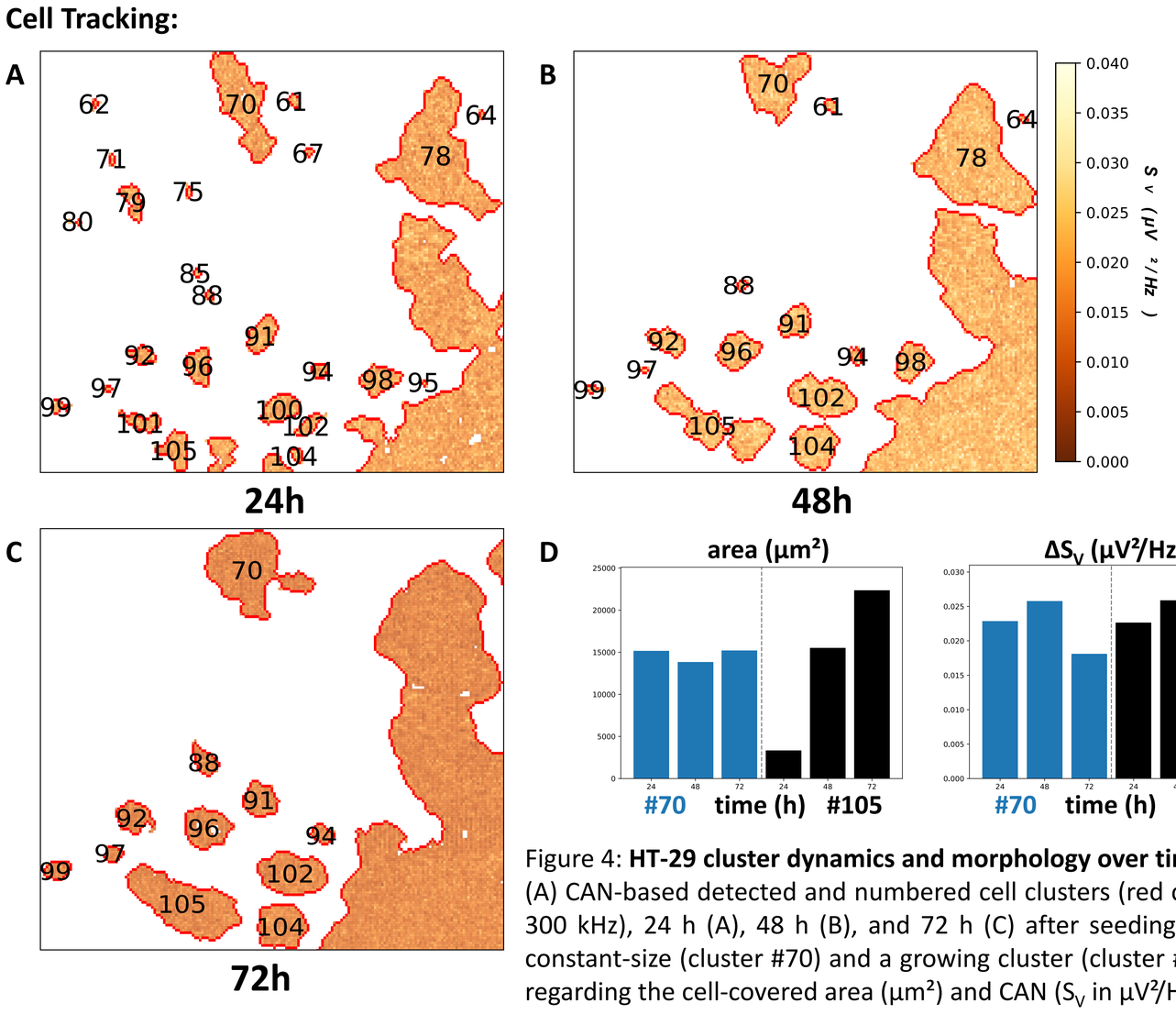

Figure 4: **HT-29 cluster dynamics and morphology over time.**
(A) CAN-based detected and numbered cell clusters (red contours, ΔS_V at 300 kHz), 24 h (A), 48 h (B), and 72 h (C) after seeding. (D) Tracking a constant-size (cluster #70) and a growing cluster (cluster #105) over 72 h regarding the cell-covered area (µm²) and CAN (S_V in µV²/Hz).

CONCLUSIONS

Adhesion noise spectroscopy constitutes a potent tool for the label-free and non-invasive cancer cell detection with high correlation between electrical and brightfield microscopy imaging. Machine-learning tools benefit from the CMOS MEA's high spatial resolution, which allows for cell type identification with high accuracy. Tracking cells and clusters over many days in culture allowed us to extract the covered area, the cell adhesion noise, or cell motility over time. Future work aims to study cancer cells' behavior after chemotherapeutic and immunotherapeutic treatment.

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