Single-cell motility analysis using nanowell-in-microwells

Pan Deng[1,2,3], Wenze Lyu[2,3], Kerryn Matthews[1,3], Simon Duffy[1,3,4], and Hongshen Ma[1,2,3]

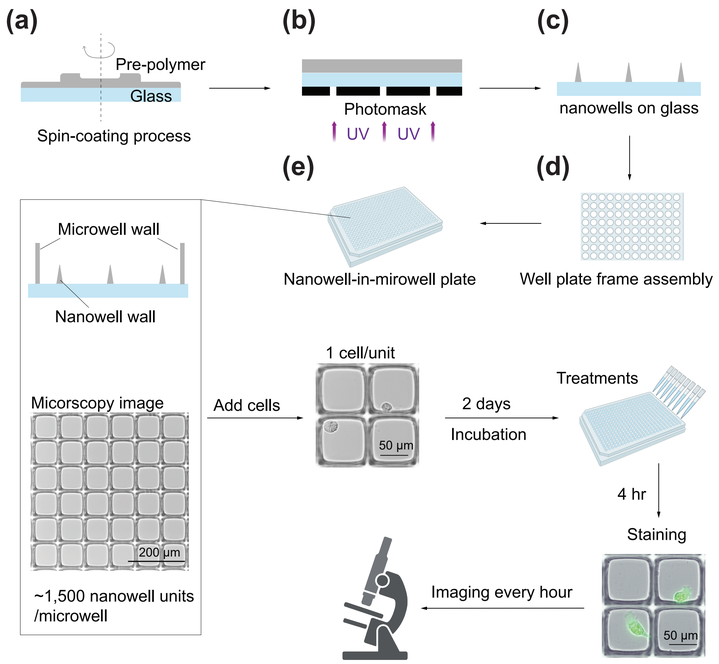
[1]Centre for Blood Research; [2]Department of Mechanical Engineering; [3]The University of British Columbia, Vancouver, BC, Canada; [4]British Columbia Institute of Technology, Vancouver, BC, Canada

Background

Metastatic cancer is characterized by the spread of cancer cells from their primary site to distant organs or tissues, significantly complicating treatment and prognosis. Various cell migration assays were devised to identify tumor subpopulations capable of initiating the tumor metastatic cascade. However, the accurate tracking of single cells continues to face significant challenges, primarily due to the limitations in technologies for single-cell isolation and tracking.

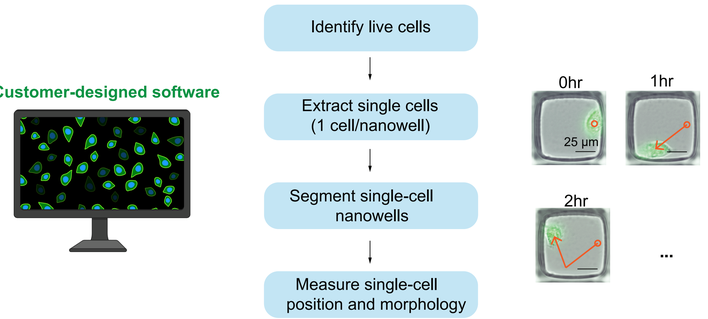
To tackle this challenge, we engineered a high-throughput assay for analyzing the mobility of individual cells. Employing this assay on breast cancer cells with time-lapse imaging at a reduced sampling rate, we systematically assessed thousands of individual cells in three different microenvironments. This systematic analysis enabled us to characterize their migration behaviors, dynamic morphological features, and mobility states, thereby shedding light on the diverse characteristics and behaviors of cancer cells during the migration process.

Methods



- Nanowell-in-microwell device fabrication:
  - (a) spin-coat a layer of UV-curable pre-polymer on a glass slide,
  - (b) pattern nanowells using UV light and a photomask,
  - (c) wash out uncured pre-polymer to leave cured nanowells on the glass,
  - (d)-(e) assemble the glass with a well plate frame.
- Nanowell dimension: 75×75×60 μm (length× width× height)
- Breast cancer cell (MDA-MB-231) seeding, treating, staining (Calcein, AM) and imaging in nanowells.
- Image data processing

Image data processing



Results

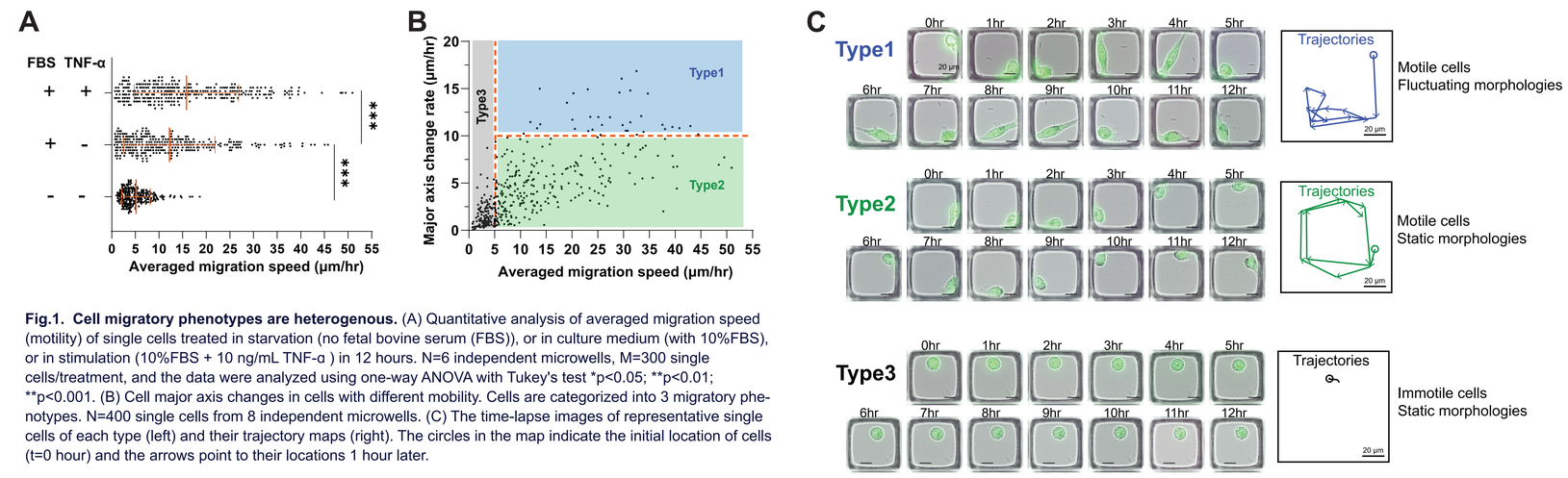


Fig.1. Cell migratory phenotypes are heterogenous. (A) Quantitative analysis of averaged migration speed (motility) of single cells treated in starvation (no fetal bovine serum (FBS)), or in culture medium (with 10%FBS), or in stimulation (10%FBS + 10 ng/mL TNF-α) in 12 hours. N=6 independent microwells, M=300 single cells/treatment, and the data were analyzed using one-way ANOVA with Tukey's test *p<0.05; **p<0.01; **p<0.001. (B) Cell major axis changes in cells with different mobility. Cells are categorized into 3 migratory phenotypes. N=400 single cells from 8 independent microwells. (C) The time-lapse images of representative single cells of each type (left) and their trajectory maps (right). The circles in the map indicate the initial location of cells (t=0 hour) and the arrows point to their locations 1 hour later.

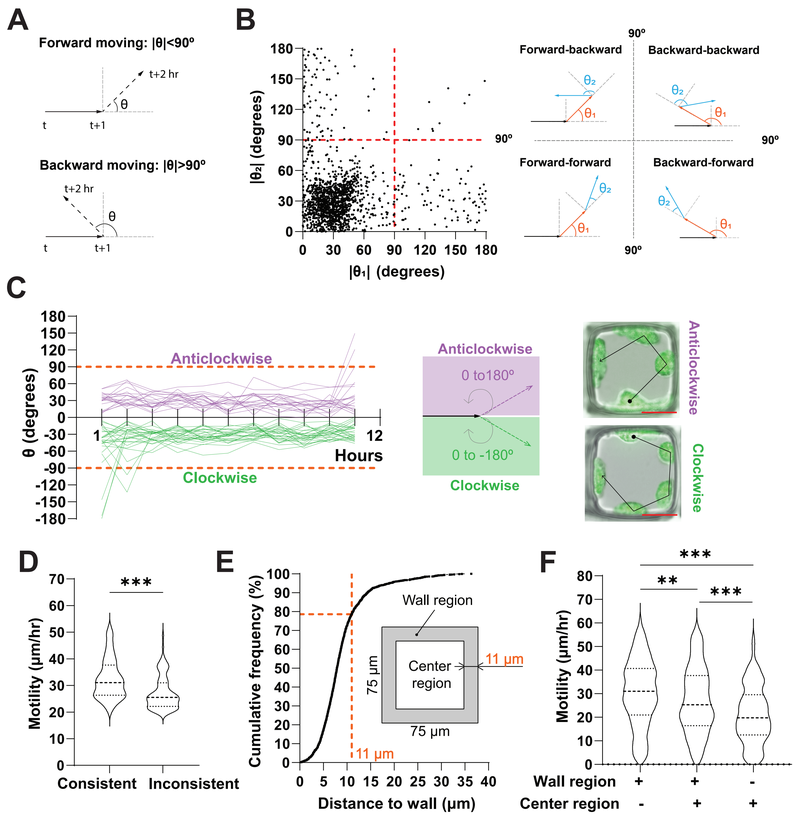


Fig.2 High-motility cells (averaged speed > 20 μm/hour) exhibit distinct preferences in migration directions and regions. (A) Schematics of the turning angle measurements to quantify cell direction changes. (B) The distributions of two consecutive turning angles ($|\theta_1|$ and $|\theta_2|$) that were measured after each time point (N=1490 data points). (C) Monitoring the turning angles in cells that consistently moved either in an anticlockwise or clockwise direction across the entire 12-hour period (N ≥ 20 single cells/group). Scale bar = 20 μm. (D) The motility (averaged migration speed) of cells migrating consistently, and the rest of cells that migrating inconsistently in 12 hours. N ≥ 50 single cells/group. (E) Cumulative frequency of the distance between cell centroids to the nearest wall of the nanowell at every time point. Inserted: Schematic definition of two regions of equal area: the center region and the wall region. (F) Cell migration speeds inside the wall region only, in the center region only, and in the transition between the two regions (N ≥ 130/group). All data were analyzed using one-way ANOVA with Tukey's test *p<0.05; **p<0.01; ***p<0.001.

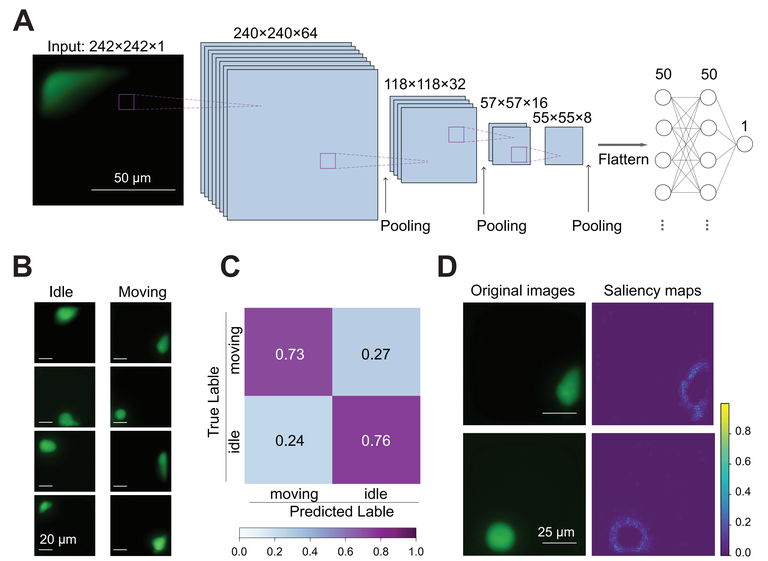


Fig.3 Visible cell features in images are associated with the cellular mobility states. (A) The architecture of the convolutional neural network model to analyze cell images. The input image dimensions, feature map dimensions and the numbers of neurons in the last two dense layers are shown. (B) Example input images of motile cells in the idle state and moving state. (C) Confusion matrix obtained from our modelling on the testing dataset. (D) Randomly selected cell images (left) and their saliency maps (right). The saliency maps highlighted the pixels that contributed to the model prediction. The strength of the contribution is indicated by the color scale.

Conclusion

- We developed a **high-throughput nanowell-in-microwell assay** and an **analysis software** to characterize the mobility and morphology features of **single cells**.
- This assay enables us to demonstrate phenotypic **heterogeneity** in breast cancer cell migration.
- Breast cancer cells exhibit distinct preferences in migration directions and regions.
- Their **cell mobility state** is associated with cellular morphology, which could be used to identify cells at idle or moving state.

Significance

We developed a single-cell mobility analysis tool capable of characterizing cell migration features, which could be further used for drug testing and cancer development predicting.