

DYNAMIC MANIPULATION AND PATTERNING OF BREAST CANCER CELLS IN BIOSOLUTION

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ABSTRACT

This paper reports the first experimental exploration of non-invasive and fast manipulation of breast cancer cells by harnessing multimode micromechanical resonators operating in biosolution. We demonstrate, for the first time, that groups of breast cancer cells are spatially manipulated into controlled microscale patterns, facilitated by the spatially abundant and diverse multimode resonances of vibrating thin micro-diaphragms. We further show that these cell patterns can be dynamically switched within 30s via programmed excitation frequencies, exhibiting a cell manipulation speed at $\sim 4\mu\text{m/s}$. The results demonstrate a versatile platform for cell manipulation and patterning at microscale, which may facilitate breast cancer related studies at cellular level.

INTRODUCTION

The ability to neatly manipulate delicate biological cells, as well as their groups at microscale precision has attracted great attention for its ample biophysical and biomedical applications, such as investigating cellular interactions (essential for cellular behavior regulation, tissue formation and cancer metastasis) [1,2], cell patterning and sorting [3,4]. Towards these goals, optical tweezers [3] are powerful for manipulating individual cells with excellent force and spatial resolutions, albeit compromised by adverse laser heating and complexity challenges when facing high cell populations. In addition to optomechanical means, employing piezoelectric devices to launch surface acoustic waves (SAWs) across cell flows in microfluidics is also being extensively studied [2,4].

Chladni figures [5], first discovered by German musician Ernst Chladni that sand particles dispersed on top of a center-clamped vibrating metal plate could aggregate into various amazing patterns in response to harmonic vibrations of the plate. This phenomenon can open up new possibilities for manipulating and patterning biological species in liquid, as we imagine scaling the classical ‘Chladni plate’ down to microscale. To date, exploiting Chladni effects at microscale has been realized to pattern microbeads/spheres in solutions, using both 1D and 2D multimode microresonators [6,7].

In this work, we take an initial step toward manipulating biological cells using Chladni multimode microresonators. This platform and the associated techniques exhibits attractive features and potential, including non-invasive and fast cell manipulation, simple device fabrication, and more importantly, spectral programmable dynamic patterning, and

spatially rich and diverse patterns enabled by the engineered multiple modes and their mode shapes.

EXPERIMENTAL DESIGN

The objective of this experimental effort is to develop a Chladni-like platform based on multimode micromechanical resonators, for collectively manipulating and patterning groups or arrays of breast cancer cells in a non-invasive and fast manner. Micro-diaphragms are chosen because of their simple geometric structure which promises facile fabrication and flat surface, and more importantly, robust presence of multiple flexural resonances even in fluidic environments. Low-stress silicon nitride (Si_3N_4) film is a good candidate for device fabrication because of its excellent mechanical and chemical properties. MDA-MB-231, an important cancer cell line (from pleural effusions) for breast cancer metastasis studies is utilized. Given the fact that these cells have an averaged diameter, $d \approx 15\mu\text{m}$ (refer to Fig. 4 for details), we design the length scale of the micro-diaphragms to be $\sim 300\mu\text{m}$ to accommodate groups of cells. In addition, given the Si_3N_4 film thickness of $\sim 600\text{nm}$, the micro-diaphragm devices are predicted to exhibit diverse flexural vibrational modes in liquid on the order of $\sim 100\text{kHz}$.

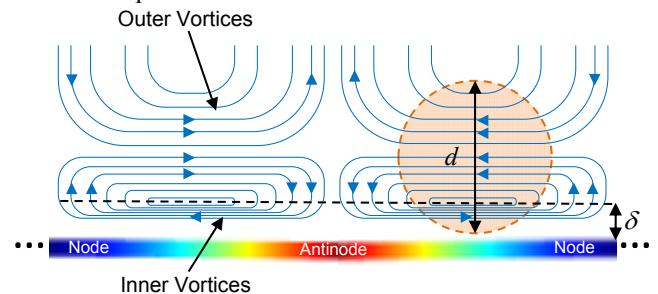


Figure 1: Illustration of the mechanism of patterning breast cancer cells in biosolution via micromechanical resonators. Boundary streaming (consisting of inner and outer vortices) induced by a resonating boundary in fluidic environment (1D beam approximation) depicts that breast cancer cells would be manipulated to antinodes given $d \gg \delta = \sqrt{2v/\omega}$, where $f = \omega/2\pi$ is on the order of ~ 100 kHz.

Figure 1 depicts the basic underlying mechanism for patterning breast cancer cells by using multimode resonators operating in biosolution. Using a 1D beam model (a

simplified case that can mimic the actual device motion from a cross-sectional view), we illustrate that the oscillating microresonator interacts with the surrounding fluid, hence ‘boundary streaming’ (vortex flows shown in Fig. 1) occurs as a result of the non-zero time-averaged fluid velocity field [6]. The streaming flows consist of both inner and outer vortices that mirror between adjacent nodes (blue) and antinodes (red), with flow directions indicated by the arrows in Fig. 1. Boundary layer thickness (δ) can be used to estimate the inner vortex height, defined as:

$$\delta \approx \sqrt{\frac{2\nu}{\omega}}, \quad (1)$$

where ν is the kinematic viscosity of the fluidic medium, and $\omega=2\pi f$ is the radial frequency of oscillation. A simple calculation assuming a micro-diaphragm vibrating in phosphate-buffered saline (PBS, kinematic viscosity is $\sim 1.1\text{cP}$) at f within 100 kHz – 1 MHz, yields $\delta \approx 1.8$ to $0.6\mu\text{m}$. These δ values are much smaller than the cell diameter ($d \approx 15\mu\text{m}$), hence breast cancer cells would be guided by the vortex flows towards the antinodal locations.

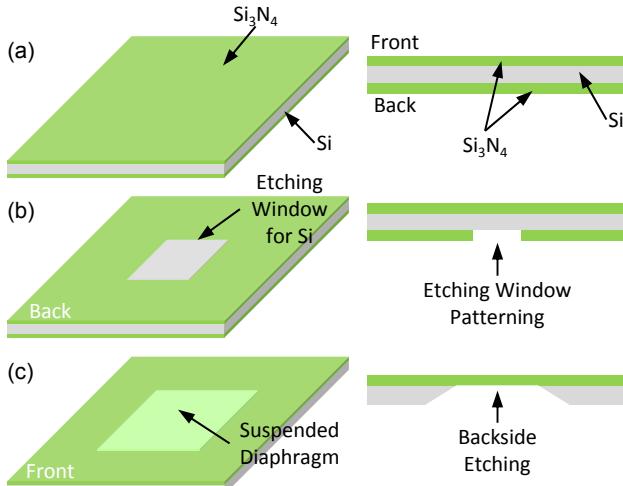


Figure 2: Fabrication process of suspended diaphragm micromechanical resonators. Aerial-view and side-view illustrations showing: (a) 600nm-thick Si_3N_4 layers are initially grown on both sides of Si wafer. (b) Selective patterning of Si_3N_4 masking layer on the backside to define etching window for Si. (c) Si TMAH backside etching. After exposing the chip to hot TMAH solution (80°C) overnight, the Si_3N_4 micro-diaphragm (length scale: $\sim 300\mu\text{m}$) is released on the front side with flat surface.

DEVICE FABRICATION

We design Si_3N_4 diaphragm micromechanical resonators (side length scale: $\sim 300\mu\text{m}$) for manipulating and patterning groups of MDA-MB-231 cells. Figure 2 illustrates the fabrication process based on Si_3N_4 -Si- Si_3N_4 wafers, with Si_3N_4 film thickness on both sides to be $\sim 600\text{nm}$. We define Si etching window by selectively patterning the Si_3N_4 masking layer on the backside (Fig. 2b). The chip is then exposed to hot TMAH solution (at 80°C) for Si backside

etching (Fig. 2c). The anisotropic nature of TMAH etching defines the diaphragm structure, and the etching time determines the lateral dimensions. Eventually, after overnight etching, we obtain suspended Si_3N_4 diaphragms with 2 types of geometries: rectangular diaphragms with lateral dimensions $\sim 300 \times 120\mu\text{m}^2$, and square diaphragms with lateral dimensions $\sim 350 \times 350\mu\text{m}^2$.

MEASUREMENT SYSTEM

To efficiently excite the micro-diaphragms’ multimode resonances in fluidic environment, we employ piezoelectric excitation [7]. A piezoelectric actuator is wire-bonded onto a ceramic package, and the circuitry is protected against the conductive biosolution by a spin-coated PMMA thin layer. Multimode resonances of a diaphragm microresonator can be excited by the driving signals supplied by a function generator. An oil-pressure controlled micropipette (refer to Fig. 3 for details) [7] enables precise delivery of the breast cancer cells onto the device surface, and the spatial distributions of the breast cancer cells are recorded in real time by an optical microscope with high-resolution camera.

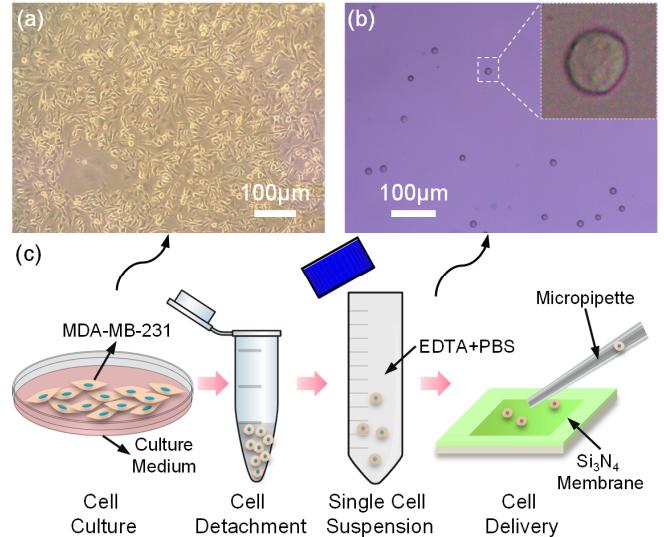


Figure 3: Breast cancer cell preparation and delivery. (a) and (b) Microscopic images showing morphology contrast of MDA-MB-231 breast cancer cells during culture and after detachment and dilution. These cancer cells become sphere-shaped and individually suspended. (c) Schematic illustrations showing the whole process of cell culture, detachment (using 0.25% trypsin), dilution (using EDTA : PBS = $10\mu\text{L} : 10\text{mL}$) and delivery (using an oil-pressure controlled micropipette, with a tip diameter $\sim 40\mu\text{m}$).

CELL PREPARATION AND DELIVERY

We culture the MDA-MB-231 cells in the medium (DMEM : FBS : Pen-Strep = $500\text{ml} : 50\text{ml} : 5\text{ml}$) for 2 days, before detaching them using 0.25% trypsin. A further dilution in EDTA solution (EDTA : PBS = $10\mu\text{L} : 10\text{mL}$) reduces cell surface viscosity, making them individually suspended. Figure 3a and 3b show clear contrast of cell

morphology and concentration before and after the cell detachment and dilution processes. Individual MDA-MB-231 cells are then locally delivered onto the surface of the micro-diaphragms by employing an oil-pressure controlled micropipette (having a tip diameter of $\sim 40\mu\text{m}$).

EXPERIMENTAL RESULTS

We first examine the microparticle manipulation and patterning capabilities of the multimode Si_3N_4 diaphragms in DI water. The ceramic package provides a ‘liquid cell’ in which device is submerged. A group of silica microbeads ($d = 3.62\mu\text{m}$) are delivered onto the device surface using the micropipette and the excitation frequency is swept within the range of 100kHz – 1MHz.

In the experiments employing a rectangular Si_3N_4 micro-diaphragm (size: $300 \times 120\mu\text{m}^2$, as shown in Fig. 4), we observe that a group of silica microbeads are manipulated into 6 one-dimensional (1D) clustering patterns, in response to the first 6 flexural resonance modes at $f=186\text{kHz}$, 271kHz , 370kHz , 500kHz , 695kHz , and 862kHz , respectively. The experimentally resolved 1D microscale patterns show good agreement with the simulation (using COMSOL). Meanwhile, the microbeads gather at the antinodal locations, which also agrees with the experimental design.

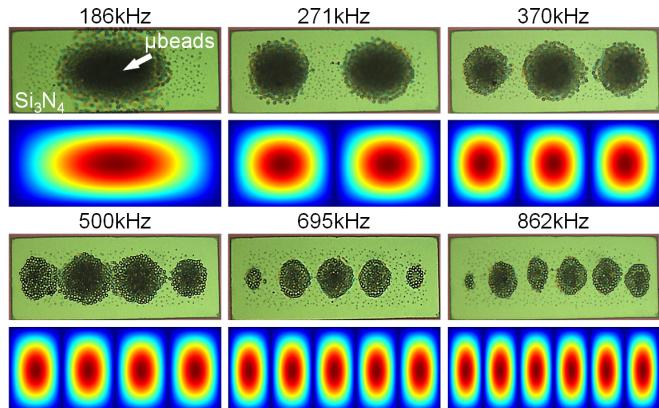


Figure 4: Patterning of microbeads using a rectangular diaphragm microresonator ($\sim 300 \times 120\mu\text{m}^2$). A group of microbeads ($d=3.62\mu\text{m}$) are observed to be manipulated into six 1D cluster patterns at 186kHz , 271kHz , 370kHz , 500kHz , 695kHz and 862kHz , corresponding to the device’s first 6 resonance modes. Mode shapes are simulated in COMSOL (red/blue colored regions represent antinodes/nodes).

In the experiments with a square Si_3N_4 micro-diaphragm (size: $350 \times 350\mu\text{m}^2$, as shown in Fig. 5), we resolve eight two-dimensional (2D) geometric patterns, in response to the diverse flexural resonances at $f=111\text{kHz}$, 210kHz , 219kHz , 239kHz , 338kHz , 422kHz , 457kHz , 546kHz , respectively. We again attain excellent agreement between experimentally resolved mode shapes and the computer simulated ones.

These results certify the microparticle manipulation and patterning capabilities of such micro-diaphragm platform. Through simply varying the device geometry, the spatially rich multiple modes are engineered in 1D and 2D fashions,

which offer excellent cell pattern diversity and flexibility.

We then examine the cell manipulation and patterning capabilities by operating these Si_3N_4 micro-diaphragm multimode resonators in biosolution. We immerse the devices into PBS solution and deliver MDA-MB-231 cells.

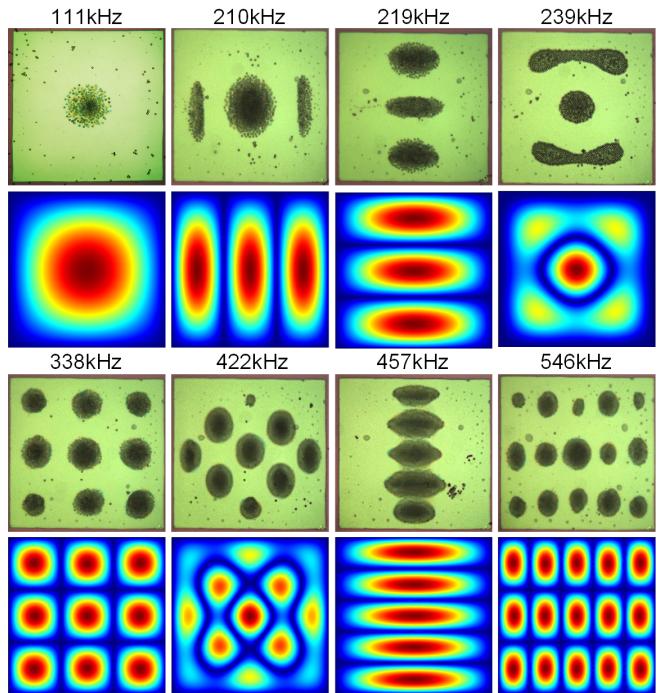


Figure 5: Microbead patterning using a square diaphragm microresonator ($\sim 350 \times 350\mu\text{m}^2$). A group of microbeads ($d=3.62\mu\text{m}$) are manipulated into diverse 2D patterns at 111kHz , 210kHz , 219kHz , 239kHz , 338kHz , 422kHz , 457kHz , 546kHz , corresponding to the device’s multiple resonance modes. Mode shapes are simulated in COMSOL (red/blue colored regions represent antinodes/nodes).

Figure 6 shows the cell experimental results with the rectangular micro-diaphragm ($300 \times 120\mu\text{m}^2$). We observe that 8 MDA-MB-231 cells are manipulated into three 1D cluster patterns, corresponding to the device’s first 3 flexural modes. These microscale cell patterns form within seconds. The time-lapse microscopic images present that by simply switching between 3 frequency values: $f_{1,3}, f_{1,2}, f_{1,1}$ (where subscripts represent the number of row and column in the ‘antinode matrix’ of a mode shape), the cell patterns can be readily altered, *i.e.*, MDA-MB-231 cells are manipulated into three clusters, two clusters, and single cluster with separation distances of 98, 147, 0 μm , respectively.

Likewise, in the experiments employing the square micro-diaphragm ($350 \times 350\mu\text{m}^2$), we observe that a total of 17 MDA-MB-231 cells are gathered into four 2D microscale patterns (as shown in Fig. 7). Switching between 4 frequency values ($f_{3,3}, f_{3,1}, f_{1,3}, f_{1,1}$) enables cell pattern transition within 30s, and MDA-MB-231 cell groups are manipulated into 9 clusters, 3 clusters, and single cluster.

To the best of our knowledge, this is the first time that fast and parallel manipulation and patterning of group of

breast cancer cells in biosolution, in both 1D and 2D fashions, is enabled by using multimode micromechanical resonators. The cell moving distances in both experiments allow us to estimate a cell manipulation speed of $\sim 4\mu\text{m}/\text{s}$.

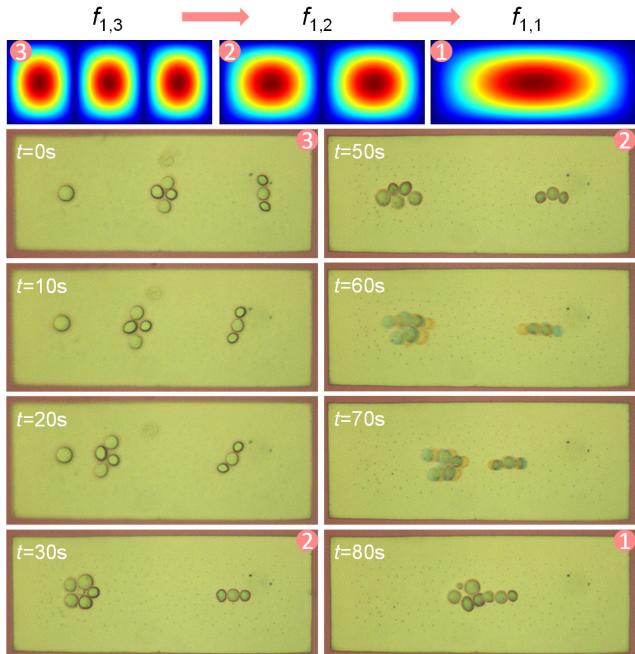


Figure 6: Patterning breast cancer cells using a rectangular Si_3N_4 diaphragm ($\sim 300 \times 120\mu\text{m}^2$) resonator. Time-lapse microscopic images show a group of breast cancer cells are swiftly controlled in a 1D fashion, into 3 patterns when the excitation frequency is switched between (1,3), (1,2), (1,1) modes. Patterns alter within $\sim 30\text{s}$, after which we maintain each pattern for 20s. Breast cancer cells aggregate at the antinodes of the corresponding mode shapes.

CONCLUSIONS

We describe the first experimental exploration of non-invasive, fast manipulation of breast cancer cells via multimode micromechanical resonators operating in biosolution. We demonstrate 2 types of Si_3N_4 diaphragm resonators (square shaped: $\sim 350 \times 350\mu\text{m}^2$ and rectangular shaped: $\sim 300 \times 100\mu\text{m}^2$), whose manipulation and patterning capabilities are first examined via experiments with microbeads. We then demonstrate that breast cancer cells are spatially manipulated into controlled microscale patterns: (i) For the rectangular diaphragm, three 1D cell patterns are resolved and dynamically switched within 30s via programmed excitation of the device's (1,3), (1,2) and (1,1) modes. (ii) For the square diaphragm, four 2D cell patterns are resolved and switched by selectively exciting the device's (3,3), (3,1), (1,3) and (1,1) modes. We further estimate the cell manipulation speed to be $\sim 4\mu\text{m}/\text{s}$. These features render our technique to be versatile in cell manipulation and patterning at microscale, without relying on the cell optical, chemical, electrical properties, which open new possibilities for breast cancer cell biophysical studies.



Figure 7: Patterning breast cancer cells using a square Si_3N_4 diaphragm ($\sim 350 \times 350\mu\text{m}^2$) resonator. Time-lapse microscopic images show a group of breast cancer cells are controlled in a 2D fashion, into 4 patterns when the excitation frequency is switched between (3,3), (3,1), (1,3), (1,1) modes. Breast cancer cells are observed to gather at the antinodes of the corresponding mode shapes.

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