Optomechanical characterization of mechanical stiffness of colon cancer cells

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Abstract

Mechanical properties of a cell reflect its physiological and pathological conditions. In this work, we improved our previous method for the optomechanical stiffness measurement of single adherent cell. The setup in this paper is using a thick silicon chip and a commercial piezo planar actuator, instead of MEMS devices as in the previous study. These changes improve the throughput and decrease the cost of each measurement. Our results on live, fixed and Noc & Cyto-D treated cells show that the proposed method can clearly distinguish them by measuring the change in the vibration-induced phase shift (VIPS). A finite element analysis model of an oscillating cell on substrate is developed to explain the measured profile of the VIPS.

Introduction

- Mechanical properties of living cells are dependent on their biological and pathological statues [1].
- Cell stiffness can be used as a biomarker for diagnostic of different disease like cancer, inflammation and blood disease.
- Various developed technologies for cell stiffness measurement can be categorized in two groups, one group works on ***suspended cells***, the other group can work on ***adherent cells***.

Suspended cell:
- Optical stretcher
- Cell transit analyzer
- Suspended micro -channel resonator
- Various other microfluidic approaches

Adherent cell:
- Atomic force microscopy
- Micropipette aspiration
- Magnetic twisting cytometry
- Optical tweezer

Limitation on adherent cell stiffness measurement :
- Most of High throughput methods require suspended cell
- Methods which do not need cell detachment have low throughput
- Stiffness measurement on a same cell with high throughput is challenging

Adherent cell stiffness measurement using VIPS :

Nano-scale vertical vibration of cell → Slight difference (~2%) between refractive index of the cell and media → Optical detection of cell's height oscillation possible by measuring VIPS → ***Cell Stiffness***

- Previously, MEMS actuator is used to vibrate the target cells, limiting the number of cells measured in each assay [2].
- A commercial piezoelectric planar actuator and a silicon chip are used, to vibrate the target cells.

Advantageous of this approach :

Increasing throughput of measurement | **Repeated measurement of same cells over time** | **Cost reduction**

Methods

- Cell on a vertically vibrating substrate experiences height oscillation.
- A Laser Doppler Vibrometer (LDV) measures the amplitude and phase of cell height oscillation.
- The amplitude and phase of cell height oscillation is inversely proportional to the cell stiffness.
- When the laser is located outside of the cell the total Optical Path Length (OPL(t)) is as follows:

$$OPL(t) = \sum n_i d_i(t) = n_{GM} * Position_{Sensor}(t) = n_{GM} A_S * \sin \omega t + const.$$

n_{Cell} :Refractive index of cell
n_{GM} : Refractive index of media
A_C : Amplitude of cell height oscillation
A_S : Amplitude of substrate's vibration
w : Angular frequency of vibration
f : Phase of height oscillation

- When laser passes through the cell, the OPL(t) will be affected by the height oscillation as follows:

$$OPL(t) = \sum n_i d_i(t) = n_{GM} * (Position_{Sensor}(t) - Height_{Cell}(t)) + n_{Cell} * Height_{Cell}(t)$$
$$\approx n_{GM} A_S * (1 + \Delta A) * \sin(wt + \Delta f) + const.$$

Apparent amplitude increase

$$\Delta A = \frac{(n_{Cell} - n_{GM}) * A_C}{n_{GM} * A_S}$$

Apparent phase shift denoted as VIPS

$$\Delta\varphi = \arctan\left[\frac{n_{Cell} - n_{GM}}{n_{GM}} * \frac{A_C}{A_S} * \sin\phi\right]$$

- The LDV laser scans a 50 µm by 50 µm area around the cell to obtain a 2-D phase shift pattern.

Cell culture:
- HT-29 cells are cultured on a bare silicon chip with DMEM growth media and 10% FBS.
- Cell fixation were done with paraformaldehyde which is known to ***increase the stiffness***.
- Noc (10ug/mL) & Cyto-D (0.1ug/mL) treatment is used to compromise cytoskeleton structure and ***reduce stiffness of cells***.

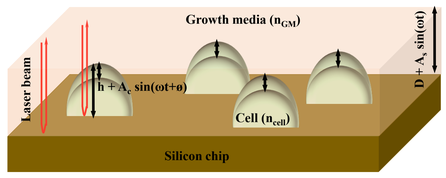

Fig1. Concept of VIPS measurement. Vibration of the cell changes the optical path length of the LDV laser, causing the apparent shift of the measured velocity's phase.

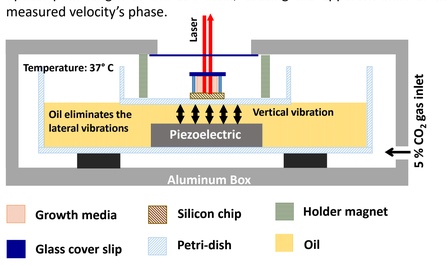

Fig 2. Culture box is made to provide an isolated environment for cells during measurement.

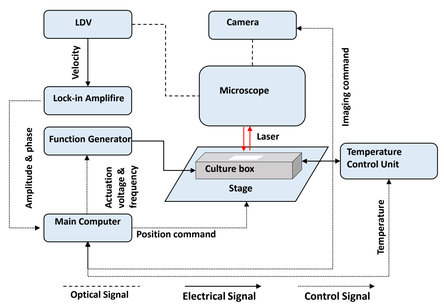

Fig 3. Schematic diagram of optomechanical cell stiffness measurement setup.

Results

2-D VIPS profile of live cell and water micro-droplet:

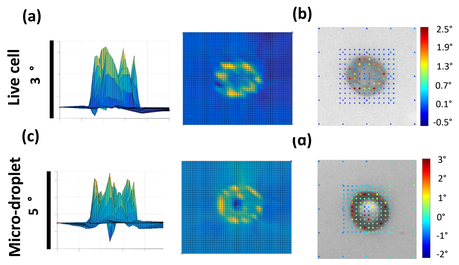

Fig 4. (a) 2-D VIPS profile of a cell and (c) a water micro-droplet. (b) Girding points specified the spots in which VIPS measurements are done on a cell and (d) a water micro-droplet.

- 2-D VIPS profile shows significant increase in VIPS on cell and micro-droplet area (Fig 4).
- Peak value of the 2-D VIPS pattern is an indicator of stiffness.
- Direct coupling of sample with PZT plate causes slope on the VIPS profile of micro-droplet. (Fig 5).

Repeatability test:

VIPS patterns for the fixed cell are consistent → VIPS Measurements are repeatable.

VIPS patterns for the live cell are not consistent → Cell morphology changes over time affect the VIPS

Direct and indirect coupling:

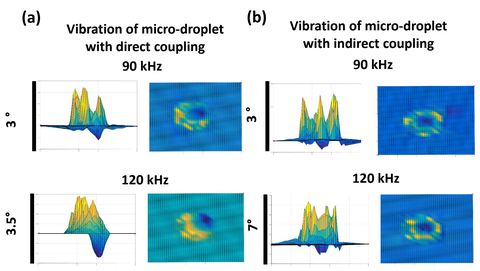

Fig 5. (a) VIPS profile of a water micro-droplet when silicon chip is directly attached to the piezoelectric plate. (b) VIPS profile of a water micro-droplet after using silicon oil to prevent lateral vibration transfer

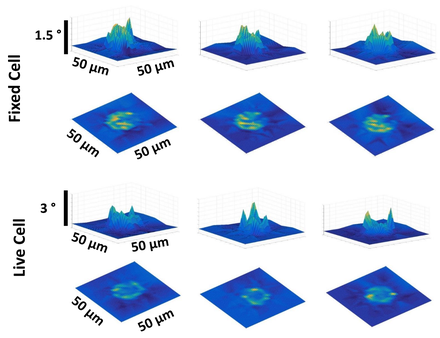

Fig 6. VIPS Measurement repeatability on a fixed and a live cell. (a) Three measurements on a fixed cell have consistent patterns. (b) Three measurements on a live cell show different patterns because of cell morphology changes during time.

Results

Cell stiffness measurement:

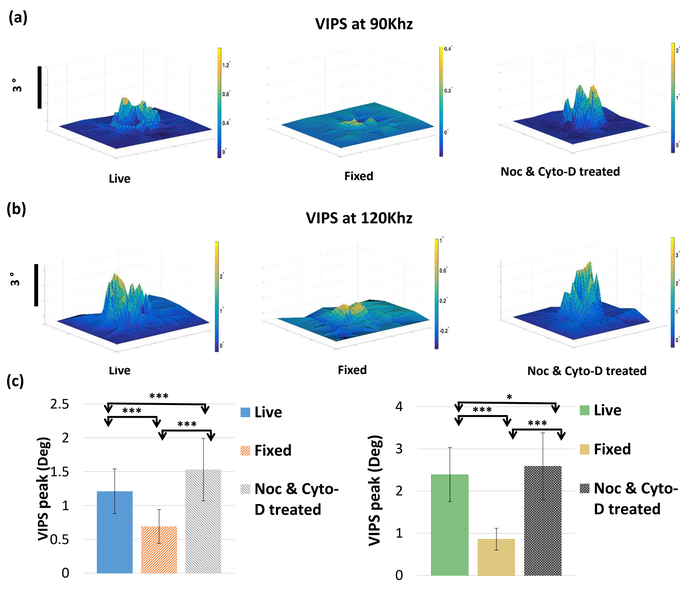

Fig 7. Peak values of VIPS on live, fixed and Noc & Cyto-D treated cells. (a, b) 2-D VIPS pattern of live and fixed cell with 90 kHz and 120 kHz vibration. (c) Average peak value of VIPS on three groups of cells. (*** means that two groups of data have statistically significant difference.)

- VIPS measurement is done on fixed (n=45), live(n=45), and Noc & Cyto-D treated cells (n=40).
- The frequency of measurement affects VIPS due to viscoelasticity of the cell.

Stiffness : **fixed cell > live cell > Noc & Cyto-D treated cell**

VIPS @ 90 kHz : **fixed cell < live cell < Noc & Cyto-D treated cell**

VIPS @ 120 kHz: **fixed cell << live cell ≈ Noc & Cyto-D treated cell**

Actuation frequency and amplitude effects on VIPS measurement:

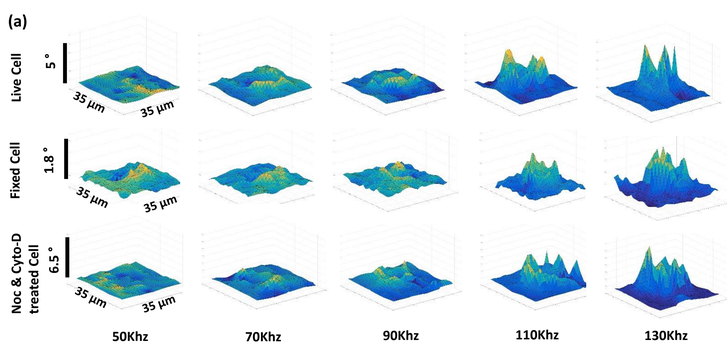

Fig.8. Effect of actuation frequency and vibration amplitude on the VIPS pattern of the cell. (a) 2-D VIPS patterns of three groups of cells in various actuation frequencies. (b) VIPS peak value vs. actuation frequency. (c) 2-D VIPS patterns of a fixed cell in different vibration amplitudes are consistent. (d) VIPS values on 5 different locations of a fixed cell in different vibration amplitudes.

As the frequency increases the VIPS peak value increases ⇔ Cell can be considered as a viscoelastic material with frequency dependent stiffness.

With increasing vibration amplitude from 0.5 nm to 4 nm, VIPS remains relatively constant, although the vibration velocity increases.

Finite element analysis and estimation of cell elasticity :

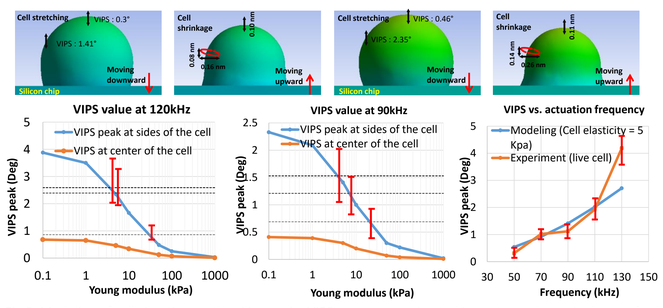

Fig.9. Modeling of cell vibration on the silicon chip. VIPS values are estimated in center and marginal area of cell at actuation frequency of (a) 90 kHz and (b) 120 kHz. (c, d) VIPS peak value versus elasticity of the cell at 90 kHz and 120 kHz. (e) VIPS peak value at different actuation frequencies.

Estimated Young's moduli:

Actuation Frequency	Live cell	Fixed cell	Noc & Cyto-D treated cell
90 kHz	7.4 kPa	27.7 kPa	4.25 kPa
120 kHz	4.8 Kpa	37.2 kPa	4.2 kPa

Conclusion and future works

- To increase the throughput and reduce the cost of optomechanical measurement of single adherent cell's stiffness, ***silicon chip*** used as cell culturing substrate and ***piezoelectric plates*** used as vibration source.
- Measuring the velocity of vibration inside and outside of the cell, a symmetric phase shift (VIPS) on the cells is observed.
- The peak of VIPS is different among live, fixed, and Noc & Cyto-D treated cells, so it is a marker of cell stiffness.
- The system is modeled in a finite element analysis software. Elasticity of each cell can be estimated from its VIPS peak value, after VIPS measurement.
- As a future work, stiffness of different types of cells in different physiological conditions should be characterized.

References

[1] Di Carlo, Dino. "A mechanical biomarker of cell state in medicine." *Journal of laboratory automation* 17.1 (2012): 32-42.

[2] Park, Kidong, et al. "Optomechanical measurement of the stiffness of single adherent cells." *Lab on a Chip* 15.17 (2015): 3460-3464.

Acknowledgement

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