SSRG International Journal of Electrical and Electronics Engineering
ISSN: 2348-8379/ https://doi.org/10.14445/23488379/IJEEE-V11I9P109

Volume 11 Issue 9, 104-111, September 2024

Original Article

A Modified Triple Coupled Cantilever for Single Cell Physical Cytometry for Mass Sensing Applications

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Received: 04 July 2024 Revised: 07 August 2024 Accepted: 05 September 2024 Published: 28 September 2024

Abstract - *The physical cytometry of single cells is indispensable in understanding different disease prognosis states in the human body. Among the physical cytometry parameters, mass plays a vital role in understanding cell behaviour, which necessitates disease diagnosis at early stages. Micro-Electromechanical Systems (MEMS) resonators have been proficient in Mass sensing applications but often hinder performance due to sensitivity and surface modification techniques, especially for single-cell studies. Mechanical coupling of these micro-resonators has shown characteristics and eigenmodes that are suitable for various applications, including mass sensing, which eliminates challenges of sensitivity. Coupled systems of cantilevers are still being researched, and two- and triple-coupled cantilevers have proven to be effective in mass sensing applications in specific second modes of excitation. In this paper, we have proposed a modified Triple Couple Cantilever (TCC) that is structurally modified for the entrapment of single cells by maintaining the coupling, for which surface modification of single entrapment is also proposed. This work is approached with finite element modelling of the device that displays conventional TCC for the degeneration of coupled eigenstates, which is achieved by $L_O/L_C = 0.3$ (overhang to cantilever length). The structural modification is achieved that can entrap single cells and demonstrate the coupling stability of the modified TCC. The mass responses of the device from a range of 10pg to 100pg for both the TCC and Modified TCC are calibrated. The mass sensitivity is achieved around 0.8×10^5 µm for a 10pg of mass addition.*

Keywords - *Coupled cantilevers, Finite element modelling, Mass sensing, MEMS resonators, Single-cell entrapment, Sensitivity, Triple coupled cantilevers.*

1. Introduction

In biological entities, cells are regarded as the basic functional units, and therefore, the behaviour of single cells is necessitated to understand cellular functions in various organisms. Among various physical cytometric parameters, mass plays a major role. In the context of debate about single-cell studies, heavier cells proliferate faster, or there are sizes independent of proliferation [1]. This conveys the exponential and linear growth models of the cell. However, most of the studies reveal that cells adopt both models during the phase of the growth cycle [2-5].

Due to chemo-mechanical stimuli of the cell's functions and checkpoints, the process of the cell growth phase is most complex and is always in the quest for study. In literature, various techniques are reported for understanding cell behaviour, growth rates, and disease prognosis using different physical cytometry of cell parameters [1, 6-11]. Among all the contemporary methods, Micro-Electromechanical Systems (MEMS) based sensors have been set forth as emerging techniques for biomolecular detection due to their feasibility of fabrication, minimal sample preparation, and need for lower sample volumes. However, MEMS-based sensors that use optical, Field-effect sensors, and Resonant beam mass sensors are prominent but have less sensitivity [10]. So, with regard to single-cell studies, sensitivity and resolution are the key challenges in the quest for study to date [12].

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The foremost challenge of conventional MEMS mass sensors using cantilevers is mass sensitivity, which changes from zero to maximum from its fixed end to free end [13, 14]. So conventional cantilevers have a limitation in the geometry that either limits the sensitivity or limits the active sensing area because of spatial non-uniformity. To overcome these limitations, the geometric transformation of cantilever structures is evident. Some of them, like pedestal mass sensors [15, 16] and suspended micro-resonating channels [10, 17], are widely adopted for mass sensing applications. Though both these devices are intended for different cell types-adherent and suspended, they have complexities in the feasibility of micro-fabrication techniques to angular orientation and submerged micro-channels.

To overcome these challenges, the use of mode localization in vibrational systems in coupled cantilevers for improving mass sensing [12] has been adopted. It is reported that coupled systems display higher order sensitivity and also offer Intrinsic Common Mode Rejection (ICMR), eliminating false positive results [18]. Among these, the major advantage of a coupled cantilever system is the detection of ultra-low mass detection, and perturbation detection can be achieved simultaneously. Moreover, the thermal stress of the cantilever when exposed to optical detection techniques is higher, which causes changes in deflection magnitude affecting the particle sensitivity because the position of the particle and deflection are co-related with thermal stress [16]. So coupled devices have been widely implemented in different applications like ultra mass sensing [12], inertial sensors, accelerometers, and electrometers [19].

This paper focuses on the development of mass-sensing devices for biological applications that use mode localization in coupled devices. There have been reported works that focus on improving the mass sensitivity of cantilevers with higher order coupling using triple couple cantilevers [18, 20-22], but neither of them discussed the methodology of biosensing or single cell mass detection mechanism in coupled arrays. Most of this reported work used PMMA beads or gold particles for the detection and determination of mass sensitivity, which does not need any surface modification or single-cell entrapment techniques. In the case of single-cell mass detection surface modification or entrapment, methods are essential for cell adhesion or entrapment onto the surface of the cantilever. With coupled cantilevers having symmetric and antisymmetric (localized) eigenstates, mass sensing can be approached by breaking the symmetry upon loading. Surface modification/entrapment needs to be maintained on all the cantilever arrays to maintain the same surface stress for biological mass sensing applications. Doing so will have the challenge of cell adhesion on one of the localized cantilevers, where the test is performed on the other hand avoiding the surface modifying the test cantilever will not yield symmetry happen.

Though the coupled cantilevers are proven to show higher sensitivity with improved resolution and specificity, they hinder surface modification techniques or entrapment methods for mass sensing in single cells in coupled mechanisms. This paper focuses on proposing single-cell entrapment techniques in coupled cantilevers for mass sensing applications to improve the sensitivity of the device.

2. Coupled Cantilevers

Micro-mechanical resonators have a large number of eigenstates, few of which are suitable for mass sensing applications. The coupling of these vibrational modes imparts new dimensions in the physical domain of resonators. For a general mechanical coupled system of bi-coupled cantilevers, which has K_1, M_1, and K_2, M_2 is the spring constant and mass of them. When both these resonators are mechanically coupled with an overhang, Kc is the stiffness.

When both the cantilevers are identical, both the stiffness and mass of individual cantilevers are equal to K and M. For Mass sensing applications, when a mass of ΔM (difference of mass concerning cantilever mass) analyte is added onto any one of the cantilevers. Then, the undamped eigenvalue can be given as Equation (1).

$$\begin{bmatrix} 1+k & -k \\ -k & (1+k)/(1+\delta) \end{bmatrix} u = \lambda u \qquad (1)$$

Where, λ represents eigenstate, δ is an effective change in the mass ΔM/M When the effective mass added is zero, it results in nonlocalized eigenmodes, which have a symmetric state in which coupled cantilevers vibrate with the same amplitude and phase. Antisymmetric eigenstate coupled cantilevers have equal amplitude with out of phase, referred to as antisymmetric phase.

Upon the mass loading, which breaks the symmetry or antisymmetric of the coupled system, in which each cantilever starts behaving, has individual systems are considered as localized, resulting in effective change in mass related to the resonant frequency of localized cantilever as shown in the Equation (2) [12].

$$\frac{\lambda-\lambda_0}{\lambda_0} = \frac{-\delta}{2} \qquad (2)$$

This equation signifies that mass sensitivity is maximum when the coupling of the system is maximum and the first mode and second modes of resonant frequency in the coupled system are very close to the conventional resonant frequency of single cantilevers. This redefines that a two-coupled system is not equally sensitive as a single cantilever, which was reported by Gil-Santos et al. [23]. But in extension of this coupling to odd number, which gives an antisymmetric eigenmode that can utilized for mass sensing with higher order sensitivity [20].

The triple-coupled system has three individual identical systems, which are mechanically coupled, yielding three independent frequencies. Where mode 1 and mode 3 are similar to two coupled systems with symmetry and antisymmetric, mode 2 is where the central cantilever does not deflect, while the other two deflect with out of phase sample amplitude deflection. A small perturbated mass addition or change in any of the outer cantilevers will break non-localization, resulting in deflection in the central cantilever. The deflection of this central cantilever is directly proportional to the effective change in mass of the system and, hence, can be used for mass detection. However, the coupled MEMS devices do have limitations of mass sensing of single cells for the stated challenges:

(1) Surface modification of active-effective sensing area, which needs to be carried out on all the individual cantilevers for achieving the coupling leaving the central cantilever also prone to cellular adhesion. This leads to the TCC being actuated in the second mode.
(2) The exact isolation of single cells on the tip of any one rear cantilever is more challenging due to the size of cells and device dimension, leading to the requirement of high spatial resolution techniques.

Both these challenges hinder the advantage of using TCC for single-cell mass sensing applications. This work focuses on modifying TCC with conventional single-cell entrapment techniques by isolating or minimizing the effect of mass addition on the central cantilever such that the amplitude of the central cantilever is proportional to mass addition on any one of the outer cantilevers.

3. Single Cell Entrapment

Cells express dynamic behaviors that might be due to intracellular noise and communications, which alter the stochastic process at the population level. In vitro environments pose more challenges to understanding cell behavior, especially for single cells [24].

The study of single-cell physical cytometric parameters like volume, mass, and stress has profound applications [25]. The TCC has proven to have better sensitivities for mass sensing applications [12, 18]. However, surface modification methods for single-cell immobilization or entrapment have gained importance because they have to maintain symmetry and retain the mode 2 characteristics for mass sensing. Single-cell adhesion is spatial dependent unless like uniform load as the deflection amplitude of the cantilever depends upon the cell mass change as well as with spatial position where the cell adhered to the surface from a fixed end [14].

Microwells have been widely used in literature due to the feasibility of pattering and reproducibility [24, 26]. Micropatterning is possible with high throughput with different dimensions and structural patterns. This work focuses on entrapment techniques that include microwell patterning.

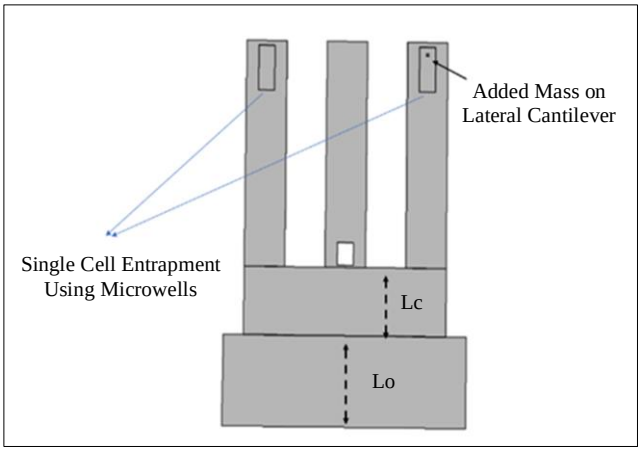

Fig. 1 Modified cantilever showing the microwells for single cell entrapment

4. Finite Element Modelling of Modified Triple Coupled Cantilevers

4.1. FEA of Conventional TCC

The behavior of TCC for mass sensing applications is analyzed and optimized for geometry, as shown in Figure 2. The Finite element simulation is performed using COMSOL Multiphysics. As reported by Pakdast and Lazzarino, 2011 [20], the dependency of coupled eigenstates and frequency on L_0/L_C, Where L_0 is overhang length and L_c is length of cantilever. For the ratio having higher 0.1 degenerate the coupled frequencies show greater frequency within modes, and less than that have very minimal or no degeneracy is visible.

In our design, we have considered the ratio $L_0/L_C = 0.3$, the frequencies of which are reported as shown in Figure 1, for other ratios of 0.1 and 0.4, where the difference between the frequency of resonant modes is very minimal or higher. Moreover, for the larger length of cantilevers with a thickness of 2 µm, the possibility of stiction plays a major role in a non-zero deflection of the central cantilever. The FEA simulation is performed with the function of added mass on TCC on one of the outer cantilevers to determine the mass sensitivity of the

device. As our studies are on single-cell analysis, a cube of 1 µm × 1 µm × 1 µm is added to the tip of the cantilever.

The mass of the cube is varied as a function of density; for example, the overall mass of the cube is varied during the simulation and does not change the dimensions meshing. This cube of mass is applied at the lateral cantilever at the extremity (free-end) to achieve the highest mass sensitivity. The density parameter (rho_d) varies from 1000 to 10000 Kg/m3 to determine the sensor's resolution.

4.2. FEA of TCC Modified for Single Cells

To facilitate the single cell mass sensing using TCC, mechanical traps/wells of 20 µm × 8 µm having a thickness of 1 µm are perforated on the lateral two cantilevers, and the center cantilever is had Stress Concentration Region (SCR) of equivalent mass which also induces higher deflection for same loading. The SCR perforations are positioned at the near end of the overhang, whereas lateral cantilevers are made on extreme ends, as shown in Figure 4. This is to maintain the mass symmetry of the cantilevers to balance them initially. To manifest single-cell mass sensing, the same cube is placed in the center cantilever microwell to understand the un-localized state of the central cantilever. The set-up experiment is sustained for multiple microwells to understand the variation of frequency and amplitude of deflection for modified TCC.

The finite element results of the micro-wells as shown in Figure 5, depict that the modified TCC is balanced with proper symmetry as central cantilever deflection in mode 2 is zero. The approach of this design pattern of well at the central cantilever close fixed end is achieved to obtain the symmetry from Equation (1). However, positioning the well in the case of the central cantilever at the rear end will facilitate the possible adhesion of particles that deviate from mode 2 for mass sensing. To void the effect of any cell adhesion on the central cantilever, the well is positioned near the fixed end of the central cantilever. The deflection of cantilever sensors is dependent upon the position of the particle from free to fixed end [14].

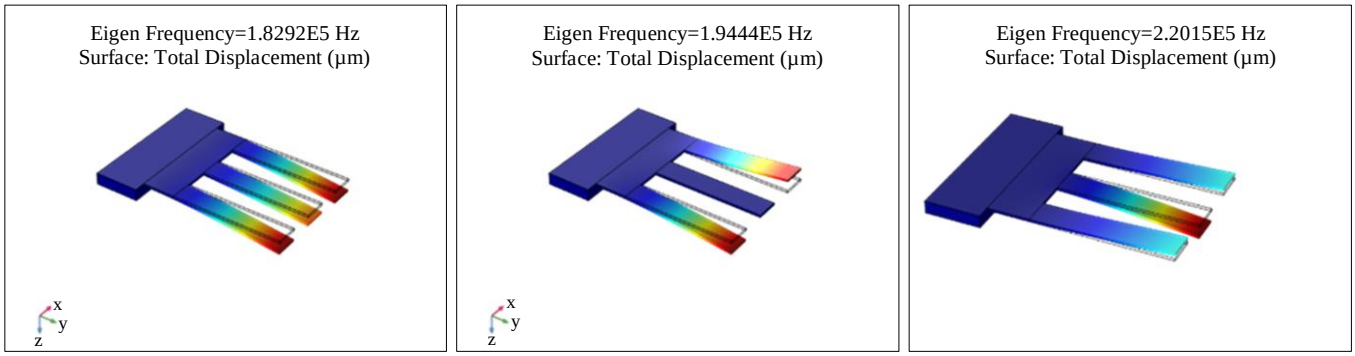

Fig. 2 (From left to right) triple couple cantilever modes where left is mode 1, centre is mode 2, and right is mode 3

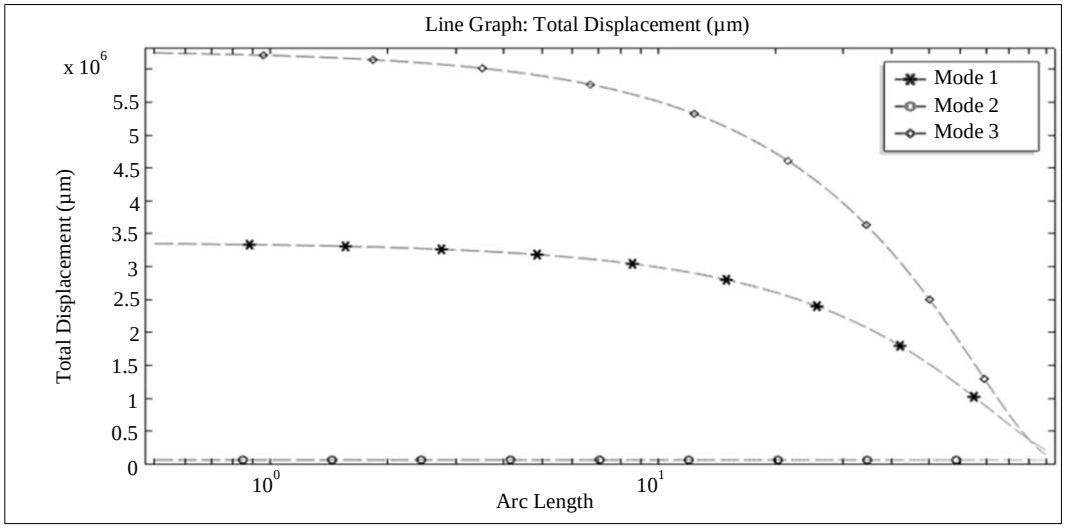

Fig. 3 Deflection of central cantilever without mass loading

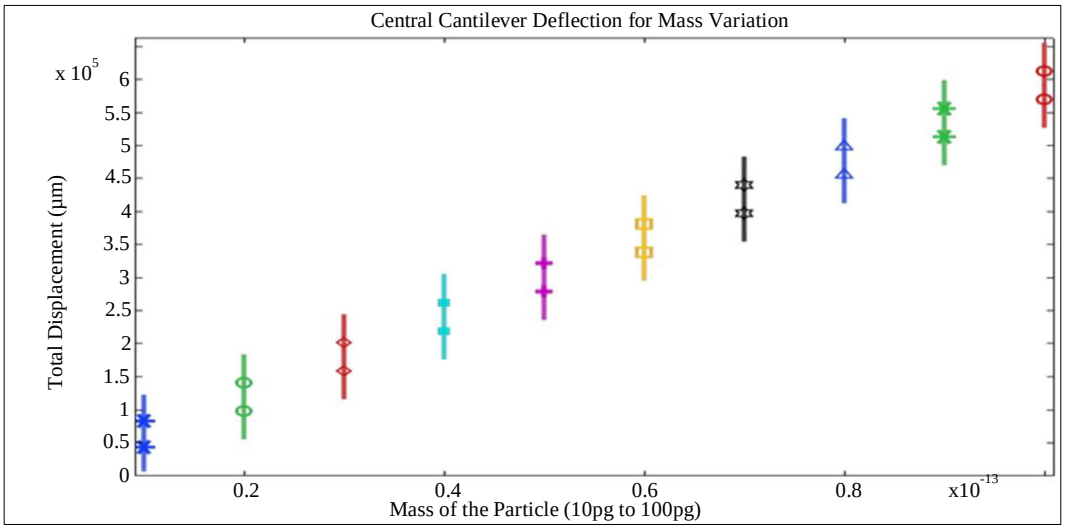

Fig. 4 TCC central deflection upon mass loading at lateral cantilever (deflection of central is increasing with increase in mass)

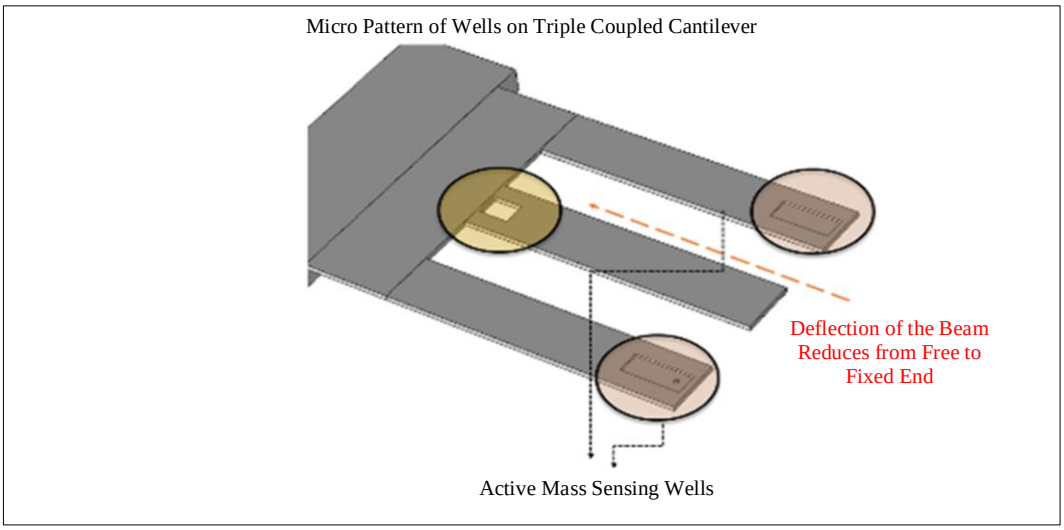

Fig. 5 Micro-patterning of wells on TCC

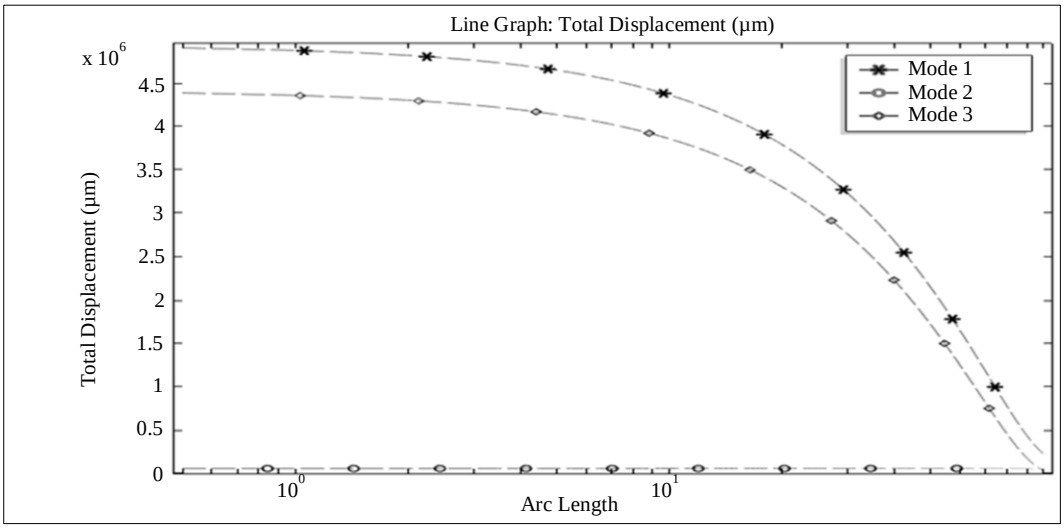

Fig. 6 Modified TCC after adding the wells for all three modes where mode 2 shows zero deflection

5. Results and Discussion

5.1. Modelling Set-Up of Modified TCC for Single-Cell Detection

A small initial mass of 50 pg is added to the microwell of a lateral cantilever of the modified TCC to determine the mass sensitivity of TCC for its deflection in mode 2, as shown in Figure 7. A parametric sweep with different masses, by varying the density of the cube, which is placed in the microwell to sweep with density as a function of mass, is shown in Figure 4 for conventional TCC and shown in Figure 8. This explains the sensitivity of the device. As there is a perforated region in the center cantilever, the possibility of particle adhesion is nullified, which facilitates the TCC and makes it suitable for single-cell studies. With the dependency of particle size and mass resolution of sensing particles, the dimension scaling of the TCC can be altered where this microwell and equivalent SCR can also be patterned.

5.2. Parametric Sweep of Modified TCC

From Figures 4 and 8, a comparison of conventional TCC parametric sweep of mass is analyzed with modified TCC, which displays the deflection of modified TCC is higher as there is a considerable amount of mass removed from the device, which increases the deflection and eigenfrequencies reported in Table 1. This can be mathematically verified from equation (2) for eigenfrequency and deflection from the equation by replacing the modified mass function ΔM/M. When compared with conventional micro-resonators, where resonant frequencies are the point of calibration for added mass, it is challenging to detect small mass changes in picograms that are less than picogram addition. Whereas in TCC, though it resonates in dynamic mode, due to mode 2 characteristics, the deflection of the central cantilever is considered for mass sensing calibration mechanism due to zero deflection (balanced state) to showing deflection in an unbalanced state (localized).

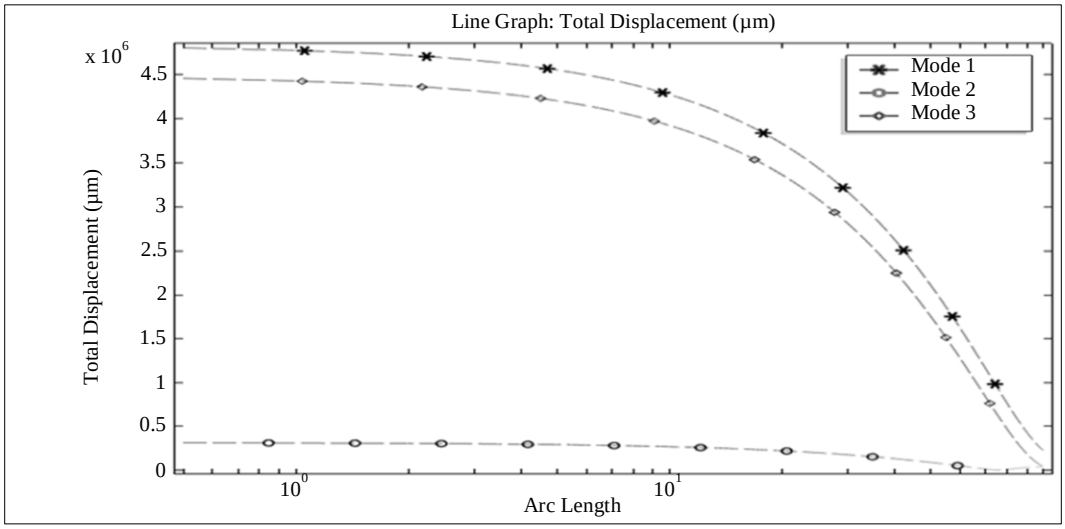

Fig. 7 Modified TCC showing deflection in mode 2 after mass addition of 50pg

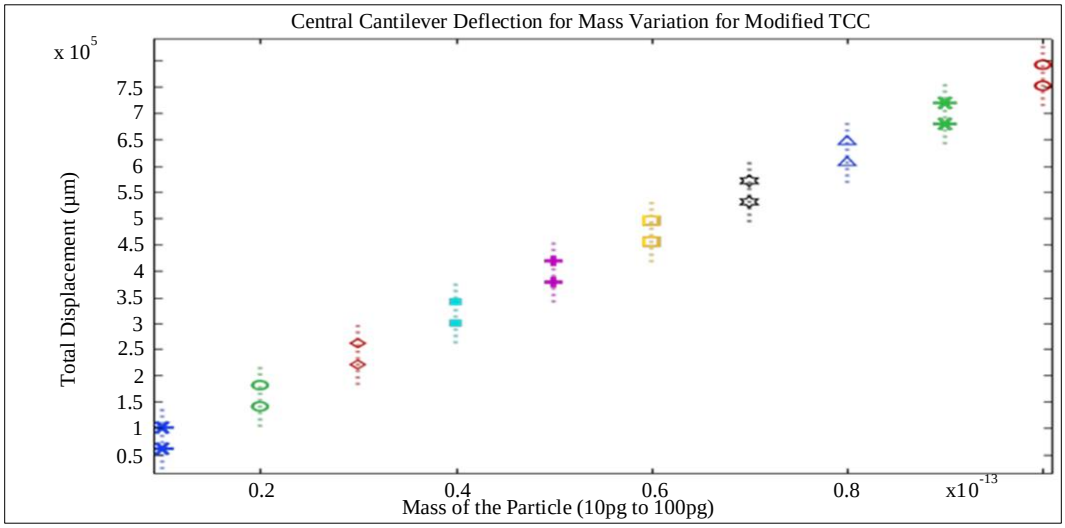

Fig. 8 Modified TCC central deflection upon mass loading at lateral cantilever (deflection of central is increasing with increase in mass)

Table 1. Deflection changes of the central cantilever for mass variation in mode 2

Mass Addition (picograms)	Mode 2 Frequency (KHz)	Deflection (um)
10	204.64	5.0×104
20	204.45	1.0×105
30	204.27	1.8×105
40	204.08	2.6×105
50	203.89	3.4×105
60	203.69	4.25×105
70	203.50	4.8×105
80	203.31	5.6×105
90	203.11	6.2×105
100	202.91	7.3×105

However, for higher resonant frequencies of mechanical structures, accurate deflection amplitude quantification is challenging. Hence, our device dimensions are chosen in such a way that the TCC resonates between 190KHz and 210KHz.

6. Conclusion

The modified triple-coupled cantilever with surface modification is adopted in this work to facilitate the single-cell entrapment. Conventional cantilever sensors have been surface-modified to facilitate single-cell adhesion, whereas coupled systems face the challenge of symmetry. This work is approached with micro-well patterning for single-cell entrapment on a lateral system and central cantilever with a perforated symmetric patch to balance the symmetric mechanical coupling. With the FEM analysis, it is observed that the TCC, in mode2, resonates at the range 190KHz to 210KHz for the mass range of 10 picograms to 100 picograms. The deflection amplitude upon the mass loading of 10 picograms is $5*10^4$ um, whereas the frequency shift is 190Hz.

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