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# Lab-on-Chip Microfluidics System for Single Cell Mass Measurement: A Comprehensive Review

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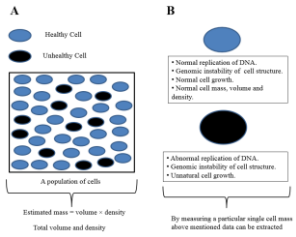
**Graphical abstract**




**Abstract**

Single cell mass (SCM) is one of the intrinsic properties of cell and is a vital part of single cell analysis (SCA). To date, a myriad numbers of works has been successfully reported for single cell mass measurement but the reported information are scattered, consequently a comprehensive review becomes mandatory to bring them together. Lab-on-chip microfluidics system integrated with micro-resonator provided an excellent platform to measure single cell mass directly (in presence of cells). On-chip microfluidics system like suspended micro channel resonator (SMR) was reported for non-adherent single yeast cell mass while 'living cantilever arrays' (LCA) was proposed to measure adherent HeLa cell mass. On the other hand, cantilever based resonant mass measurement system has non-uniform mass sensitivity; this issue has been overcome by pedestal mass measurement system (PMMS). PMMS has a unique geometrical structure that provided uniform mass sensitivity to the sensing surface. Moreover, we presented a comprehensive discussion of each of the available methods of SCM elaborating the sensing mechanism, geometry of the sensor and governing equations. It is hoped that, information presented in this comprehensive review paper will be a valuable source for the single cell mass analysers and biological researchers.

*Keywords*: Single cell mass; suspended micro channel resonator; living cantilever arrays; pedestal mass measurement sensor



## 1.0 INTRODUCTION

With the revolution of micro-bio and nano-bio technology, physiology of single cell is being discovered day by day. Great strides have been taken to develop the technology to investigate the intracellular and extracellular properties of single cell. For example analysis of single cell inside environmental scanning electron microscope (ESEM) [1]–[4], AFM cantilever for single cell strength analysis [5], Nano scale electrochemical probe for single cell analysis (SCA) [6], SCA through electrochemical detection [2], [6]–[11] and microfluidics disk for single cell viability detection [12]. SCA elucidates complex cellular functions such as cell's mechanical, electrical and chemical properties. Single cell mechanics is one of the vital part of the single cell analysis. Recent development of micro electro mechanical systems (MEMS) provide an excellent platform to analyse single cell mechanics often known as lab-on-chip (LOC) microfluidics device [7], [10], [12]–[16]. Studies on cell mechanics acquired a great interest of scientist as cell mechanics can be related to the early diagnosis of disease through single cell surgery and cell wall stiffness [17]–[20]. Cell mechanics consist of (but not limited to) cell wall strength, cell mass, density and volume at different phases of cell growth cycle. However, in this comprehensive review article we limit our scope to single cell mass and its measurement techniques. Previously, a few illuminating review papers were presented reflecting chemical and biological analysis of single cell [21], single cell analysis for quantitative biology [22], single cell trapping mechanisms [23], single cell in biotechnological applications [14] and single cell culturing methods. Nevertheless the review on single cell mass (SCM) measurement is yet to be done and technological advancements of SCM remains scattered. In order to bring this scattered information in a single platform we have presented in this review article.

Lab-on-chip integrated with microfluidics system enabled scientist to measure the mass of individual cells directly (in presence of alive cells). Micro-nano mechanical resonators have opened the doors for single cell mass measuring with high accuracy. Frequency of the resonator is inversely proportional to the acquainted mass of resonator [5], [24]–[28]. Using this principle Burg *et al.* proposed suspended micro channel resonator (SMR) for single cell mass measurement [7], [29]. But this work was limited to dry cell only i.e. non adherent yeast cell [13], infect micro beads was used to characterize the sensor [30]. This issue has been overcome by 'living cantilever arrays' for measuring adherent HeLa cell [10]. However, resonating cantilever has non-

uniform mass sensing ability [31] as a result accuracy depends on the cell position merely [10]. On the other hand, cantilever based mass measurement techniques were not able to relate the cell mass with cell growth through cell cycle. These issues have been addressed by object position independent pedestal mass measurement [16]. Results showed that, cell mass increases exponentially with cell growth which is in agreement with the previous arguments [16], [32].

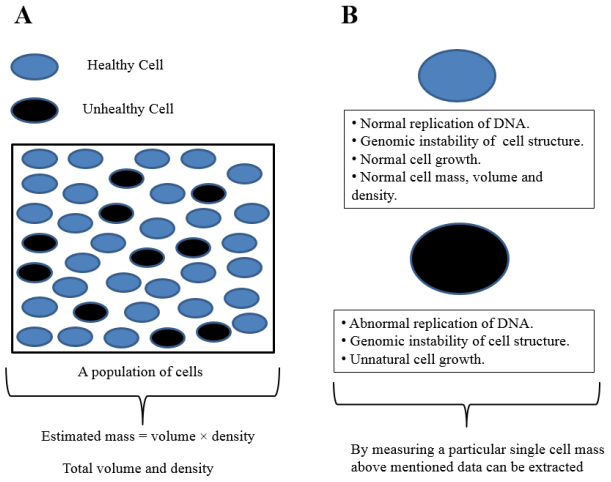



**Figure 1** (A) A large population of cells. (B) Properties of particular healthy and unhealthy cell

### 1.1 Significance of Measuring Single Cell Mass

Cell mass depends on the synthesis of proteins, DNA replication, cell wall stiffness, cell cytoplasm density, cell growth, ribosome and other analogous of organisms [10]. Chronic diseases like cancer and tumour affect intracellular physiological properties of cells [33], subsequently cell mass and density will be changed as well [32], [34]. Single cell mass can be measured either from an absolute single cell or from a large population of cells. But from the average data of single cell mass, it is not possible to identify the mass of a particular single cell. Hence, ambiguities arise in identification of cell's physical data. On the other hand, an absolute single cell mass data is able to explain the physical conditions of a particular cell. This leads to differentiate an unhealthy cell from a bunch of healthy cells and vice-versa for the healthy cell. Figure 1 shows a concept, how single cell mass contributes in terms of identifying infected cell. Figure 1A describes a schematic diagram of a population cell. From the estimated data of population cell mass, it is not possible to differentiate the healthy and infected cell. On the other hand, Figure 1B shows the single cell property for a particular cell only. For an infected cell, all these internal particles are being affected by the foreign agents or materials. These physiological changes also affect the mass of single cell. For example, in a tumour infected cell, integrity of DNA faces continuous challenges and genomic instability occurs to the chromosome's structure [35]. Inevitably, this will cause severe change to DNA replication, cytoplasm density and cell volume which ultimately leads to the changes in single cell mass. In this condition, if we could determine the mass of a single cell, we will be able to differentiate the unhealthy cell from healthy cells by investigating single cell mass property. Single whole cell mass has also a great contribution in terms by generating biomarkers for rapid identification of intact microorganisms like virus and bacteria [36]. As a result, we strongly believe that studying single cell mass and its measurement techniques will enhance our understanding of physiological properties of cell and perhaps it may provide new tools for disease diagnosis through the variation of single cell mass property of identical cells at different health conditions.

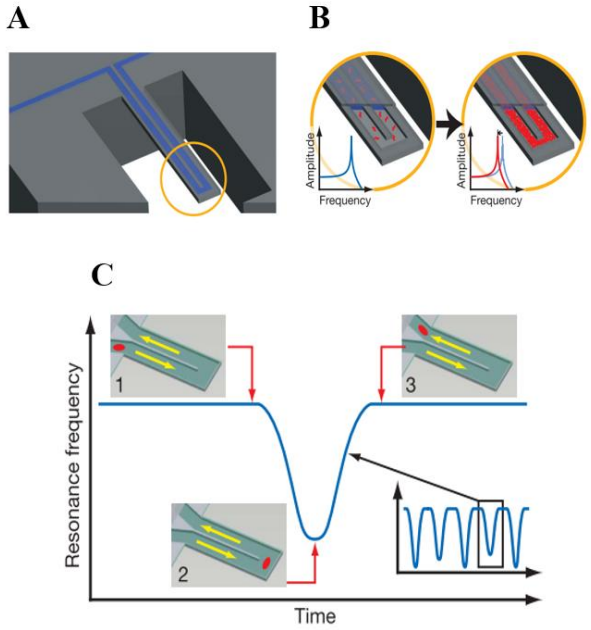



**Figure 2** (A) A typical SMR, where cantilever is hanging. (B) Frequency shifting in presence of cell. (C) Frequency shifting of the cantilever at different position of the cell [7]

## 2.0 LAB-ON-CHIP SUSPENDED MICROCHANNEL RESONATOR (SMR) FOR SINGLE CELL MASS MEASUREMENT

Suspended micro channel resonator (SMR) was proposed by Burg *et al.* for bio molecular detection from the frequency shifting of cantilever in 2003 [29]. Although this technology was initially proposed for bio molecular detection, its area of application has subsequently expanded into single cell mass and density measurements [7], [9], [13]. Frequency of the resonator is inversely proportional to the square root of its mass [7], [9], [10], [29]. This principle enables frequency shifting cantilever to measure the single particle mass in 100 mg level with high quality factor i.e. adequate sensitivity to detect each and every particle. Figure 2A shows a schematic of typical SMR, where the cantilever is at the hanging position and to be resonated. An electrostatic actuator is placed at the bottom of the cantilever to generate sufficient actuation ('DC' voltage ~60). This actuation generates micro-newton centrifugal force, which provides excellent mass sensing capability to the cantilever. At the presence of any particle, the frequency generates pick value and each of the pick is inversely proportional to the square root of current mass of the cantilever [7]. Relationship between mass and frequency is illustrated in Equation 1 proposed by Sarid [37]. Relation between the particle mass and frequency depends on the position of the particle on the cantilever. The maximum experienced mass by the cantilever is when the particle at its apex. Schematic at Figure 2B illustrates this phenomenon of mass sensing by the resonating cantilever. Figure 2C illustrates a more specific relation between mass and frequency of the cantilever. Frequency of the cantilever was measured by a position sensitive photo detector (PSD).

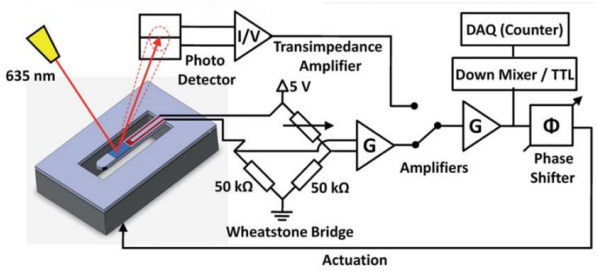



**Figure 3** Frequency detection mechanism with electronic (Wheatstone bridge) and optical detectors. Phase shifter enables feedback actuation [15]

$$f = \frac{1}{2\pi}\sqrt{\frac{k}{m^* + \alpha\Delta m}} \quad (1)$$

Where, $f$ is the resonating frequency, $m^*$ is the effective mass, $k$ is the spring constant, $\alpha$ is the numerical constant that depends on the added mass to the resonator $\Delta m$. When measureable particle is at the apex numerical constant $\alpha=1$. Cantilever is coated with 100 nm Aluminium thin film to obtain a good electrical conductivity and a high optical reflectivity [29]. Smallest change of the frequency can be determined from the ratio between the surface areas to the total mass as shown in Equation 2. Optimizing this ratio is known as surface absorption [7], [9].

$$\frac{\Delta f}{f} = -\frac{1}{2}\left(\frac{A}{m}\right)\Delta\sigma \quad (2)$$

Where, $\Delta f/f$ is the related frequency shift, $A$ is the area of the cantilever, $m$ is the loaded mass and $\Delta\sigma = A/\Delta m$ is the surface mass loading. By increasing surface area to mass ratio, the mass sensing resolution of the cantilever can be enhanced.

### 2.1 Optimization of the SMR Design for Single Cell Mass Measurement

SMR was fabricated by etching microfluidic channel on a standard silicon wafer using photolithography and reactive ion etching (RIE). For a fine detection of biomolecules, suspended micro channel must be thin and the channel should have the capability to flow the liquids continuously. Keeping these purpose ahead, SMR was fabricated with channel wall thickness of 800 nm and a fluid layer of 1.2 µm thick. Combination of polysilicon Damascene and sacrificial layer [38] etching in bulk micromachining of hot potassium hydroxide [39] was used to fabricate the suspended micro channel resonator. Actuation was generated through electrostatic force and optical lever used to detect the resonance frequency. Recently, SMR was modified with piezoresistive actuator by Lee *et al.* [15] and the frequency detection mechanism has changed to electrically instead of optically. Optical detection of frequency has two major limitations; external laser source required to generate the optical beam and a photodiode detector to detect the frequency shift. Multiplexed measurement was not possible with the SMR that proposed by Burg *et al.* Figure 3 shows SMR's frequency detection mechanism with both optical lever detector and electronic detector modified by Lee *et al.* Three external resistors were combined with on-chip piezoresistor to build a Wheatstone bridge. The output signal passes to the amplifier either through the Wheatstone bridge or photodiode. Phase shifter determines the frequency shift and feedback the signal to the actuator. This configuration of SMR, allowed it to be a feedback suited actuator and able to detect the dynamic displacement of the SMR [15].

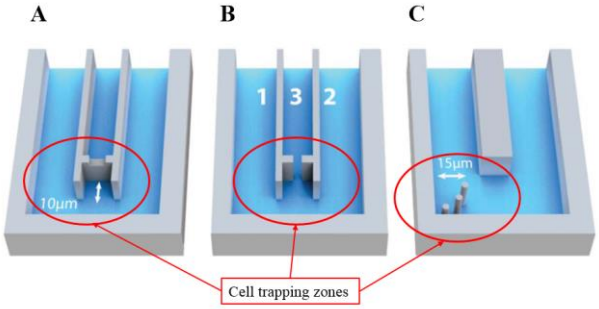



**Figure 4** Top view of the mechanical trap using SMR. (A) SMR with 3×8 µm channel and 200 nm horizontal slit. (B) SMR with 8×8 µm channel and 2 µm vertical opening. (C) SMR with 15×20 µm channel and three columns with even diameter or 3 µm. Red circle is the trapping zone for each cantilever [30]

Weng *et al.* remodelled the SMR with three channels and columns configurations [30] so that single cell can be trapped inside the fluidic channel. Besides that, there are several cell trapping methods available such as dielectrophoresis [40], hydrodynamic cell isolation arrays [41], optical tweezers for cell trapping [42] and also acoustic effect for cell trapping [43]. But these methods were limited to cell trapping only while recently modified SMR has an integrated cell trapping system for single cell mass measurement. The fabrication process was described elsewhere in [7], [29]. There were two types of the three channel SMR were fabricated, one was 3×8 µm device with 200 nm horizontal slit [Figure 4A] between the channel and another was the cross-sectional area of 8×8 µm and 2 µm wide [Figure 4B] vertical opening [30]. Three channels SMR trap the cell at the apex of the cantilever which ensured the maximum mass sensitivity of the cantilever [7]. For the columned SMR, the cross-section area for the channel was 15×20 µm and three columns (3 µm diameter for each column). Each of the columns was separated evenly in a gap of 3 µm [Figure 4C]. These columns can be placed either at the corner or centre of the cantilever, depending on the user demand. This type of configuration enables the suspended micro channel to trap single cell and perform the desired measurement. Again Arlett *et al.* proposed that by integrating SMRs with large number of arrays mass resolution can be improved significantly. This configuration of SMR is able to detect proteins and other rare biologically important particle like *virions* [44]. Table 1 summarized the configurations of SMR modified by different authors.

### 2.2 Single Cell Mass Characterization Using SMR

SMR detects any change in mass of the cantilever and translates to resonant frequency. Single cell characterization using SMR had two approaches. One is to estimate the single cell mass from a known number of population cells and another is to measure the single cell mass for a particular cell only. In 2009, Bryan *et al.* has initiated the first approach to measure the buoyant mass of a population of cells. Buoyant mass is the mass difference between cell and displaced liquids on the cantilever [13]. This buoyant mass caused the frequency changes of the cantilever as well. Frequency of the cantilever is inversely proportional to the square root of its total mass as illustrated in Equation 1. A commercial coulter counter was used to estimate the volume of cell. For large

number of sample (n > 20,000), volume of single cell was estimated as 27.0 ± 0.1 μm$^3$. Relation between buoyant mass and volume were illustrated in Equation 3.

$$m_b = V\left(\rho_f - \rho\right) \quad (3)$$

Where $m_b$ is buoyant mass of cell, $V$ is volume, $\rho_f$ and $\rho$ are the density of fluid and cell respectively. From Equation 3 average single yeast cell mass was calculated as 1.38 ± 0.010 pg [13]. As single cell volume was estimated from the total size of the population and so for the density. This average estimated data was unable to illustrate the mass of a particular single cell, subsequently real time mass data of each cell remains elusive.

**Table 1** SMR with different configurations

| Authors | References & Year | Configuration | Frequency detection methods | Remarks |
|---|---|---|---|---|
| Burg *et al.* | 2003,2007 [7], [29] | U-Shaped | Optical lever | SMR was used to detect biomolecules and estimate single cell mass from average data |
| Lee *et al.* | 2010 [15] | U-Shaped | Optical lever and piezoresistive | Feedback suited actuator |
| Weng *et al.* | 2011 [30] | Three channel and columned | Piezoelectric and optical | Cell trapping mechanism has been added |
| Arlett *et al.* | 2010 [44] | Double clamped beams | Thermoelectric actuation and piezoresistive detection | Flow through detection methods introduced |
| Lee *et al.* | 2011 [45] | U-Shaped | Photo detector | Second flexural bending mode to lower the minimum detectable particle size |

In 2011, Grover *et al.* has overcome this issue by measuring buoyant mass [46] of a particular single yeast cell using SMR. Archimedes' (at 250 B.C.) theory was used to measure the buoyant mass of the particle. Equation 4 illustrates the relation between particle buoyant mass, absolute mass and fluidic density. By measuring the buoyant mass of a particle in two different medium of known density, Archimedes was able to calculate the density of the crown (particle). The same approach was used by Grover *et al.* to measure the density of a particular single cell.

$$m_b = m\left(1 - \frac{\rho_f}{\rho}\right) \quad (4)$$

Where, $m_b$ is the buoyant mass of the particle, $m$ is the absolute mass of particle, $\rho_f$ is the density of the fluid and $\rho$ is the density of the particle. This method of single cell mass measurement required two major mechanisms. One is a high resolution mass sensor and a way to change the fluidic flow direction as faster as possible. Initially, cantilever was filled with liquid which was less dense than cell (red, step1) and the density of the fluid was measured from resonant frequency of the SMR [Figure 5A]. At the next step, cell was passed through the channel and from the pick (step 2) of resonant frequency buoyant mass of cell was measured. The direction of the flow was then reversed and cantilever was filled with high dense fluid (blue, step 3). As the blue fluid was heavier than the red, this causes frequency drop. Similarly step 4 was performed to measure the buoyant mass of cell at high dense fluid. From these four steps, absolute mass of single cell and density was calculated using Equation 4. This relationship between absolute mass and buoyant mass has been elaborated graphically in Figure 5B.

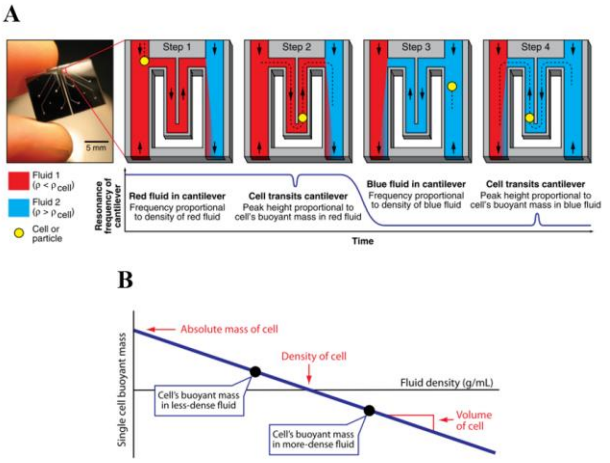



**Figure 5** (A) Steps of measuring single cell buoyant mass. Red circle on the frequency curve indicates the frequency drop due to cell in red (less dense) fluid. Blue circle on the frequency curve indicates the frequency drop due to cell in blue (dense dense) fluid. (B) Relation between buoyant mass and absolute mass of single cell [46]

However, we observe that many researchers have reported SMR as an effective on-chip device for characterizing single cell mass and density. Yet, there are some limitations and ambiguities remain. For example, cell viability, cell adherent properties, cell sorting mechanism and mass sensing error which could be up to 100% for a suspended cantilever mass sensor [16]. By improving aspect ratio of the cantilever sensitivity error can be minimized [10]. On the other hand, position dependency results of the SMR can be improved by adding flexural bending ability to the cantilever [45]. Single cell sorting is also one of the toughest

challenge in SMR mass measurement sensor. In the structure of a SMR, micro channel is hanging with an opening inlet and outlet. In order to pass a cell through the suspended micro channel, cell must be injected through the gate of the inlet [23]. There might be two possible way to manage the single cell to pass through the channel [14]. One is to sort the cell in another lab-on-chip and then inject to the suspended cantilever beam. Another approach might be, to use a valve analogous gate at the inlet of the channel which will allow only a single cell at one time to pass through the channel. After all, a micro-nano manipulator can be used for positioning the single cell in the inlet of the channel [21]. As a consequence we may conclude that, SMR can be modified with additional feature to obtain an optimised sensor for measuring single cell mass. For instance, introducing of dynamic 'mass-spring-damper' model to extract the spring constant of the cantilever and minimize the object position dependency [16].

## ■3.0 'LIVING CANTILEVER ARRAYS' (LCA) FOR MEASURING SINGLE CELL MASS

Even though suspended micro channel resonator has a great contribution to the advancements of single cell mass measurement techniques, yet this method is limited to non-adherent cell only [16] and cell stiffness data remained elusive. But it has been believed that, cell stiffness is a significant parameter that impact on stress-induced cell spreading [47], cell differentiation [48] and cancer metastasis [8]. As a consequence, Park *et al.* has proposed 'living cantilever arrays' to measure adherent cell mass [10]. In this method, cantilevers were submerged into the L-15 growth medium and cells were cultured. Hence, cells remain alive and adherent cell mass was measured using cantilever arrays mass measurement sensor. Figure 6A shows the schematic diagram of the cantilever arrays. Laser doppler vibrometer (LDV) was connected with the cantilever to measure the vibration frequency precisely. Positive dielectrophoresis was used to attach the cells on the cantilever. Frequency shifting phenomenon was used by Park *et al*. to relate frequency with cell mass. Similarly like SMR, the cantilever array used the frequency shifting phenomenon at the cantilever beam. Mass changed $\Delta m$ was measured from the frequency shifting [49] as illustrated in Equation 5.

$$\Delta m = \frac{k}{4\pi^2}\left(\frac{1}{f_1^2} - \frac{1}{f_0^2}\right) \quad (5)$$

Where, $f_1$, $f_0$ is the resonant frequency with and without cell respectively, $k$ is the spring constant of the cantilever. For a cantilever, typical equation for spring constant is denoted by Equation 6.

$$k = E\frac{t^3 b}{3L^3} \quad (6)$$

Where, $E$ is the elastic modulus, $L$ is the length, $b$ and $t$ is the width and thickness of the cantilever. The cantilever arrays were named as "living cantilever arrays" as cells were captured and cultivated on the surface of the cantilever, which kept the cell alive and adherent properties unchanged.

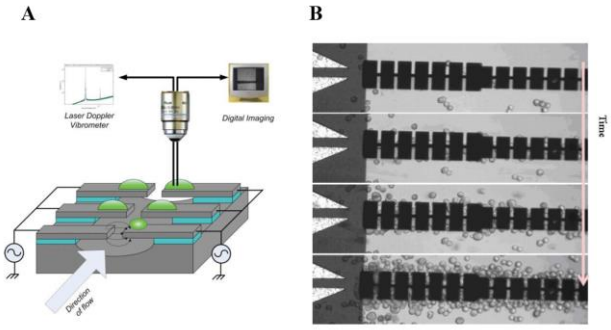



**Figure 6** (A) Living cantilever arrays for single cell mass (SCM) measurement. (B) Cell capturing using dielectrophoresis (DEP). DEP input signal was 6 Vpp at 1 MHz [10]

### 3.1 Fabrication and Characterization of LCA

'Living cantilever arrays' were fabricated from a silicon wafer. Length of the cantilever was varied from 25-40 µm long, 10 µm wide and 240 nm thick. Shortest cantilever was 25 µm long and longest was 40 µm. Initially, silicon wafer was a 500 µm substrate with 240 nm thick device layer (Figure 6A). Desired dimensions of the cantilever were obtained by reactive ion etching (RIE). Cantilever's electric conductivity was enhanced by implantation of boron at 10 KeV, it was then further tempered at 900°C for 30 min. A PDMS slab with a 2.5 mm wide and 250 µm high microfluidic channel was fabricated to cover the silicone cantilever. Thermal noise was the excitation source and an advanced Fourier transform was performed to produce sufficient vibration. Frequency of the cantilever was detected with a LDV (Laser Doppler Vibrometer, MSV-300, Polytech PI).

### 3.2 Cell Capturing and Culturing on LCA

The aim of this work was to measure the single cell mass without distorting the adherent properties of cell. Positive dielectrophoresis (DEP, 6 $V_{pp}$,1 MHz) was used to capture the cell from liquid medium. Biomolecules have dielectric property, which allows DEP in many medical applications like bio molecular separation, isolation etc. [50]. Silicon cantilevers were coated with poly-L-lysine and poly-L-lysine become highly positive at the physiological conditions. Cells were captured by the cantilevers due to the electrostatic interaction. Figure 6B confirmed cell capturing using DEP. With increasing of time, number of attached ells also increased. Once cells were captured, DEP signal was turned off. Cell growth media was inserted immediately to keep cells alive. As there is no DEP at this stage, cells were attached to the cantilever due to the adhesion property of cells only. Immortal human cervical cells, HeLa were used as a sample cell. HeLa cells culturing required 3-7 days to observe the cell growth properly.

### 3.3 Single Cell Mass Characterization Using LCA

After capturing the cells, cells were cultured inside incubator at 37°C and 5% $CO_2$ and cells growth was observed for several days. After several days (up to 7 days) of cell culturing, frequency of the cantilever was measured with LDV. Later cells were detached from the cantilever by trypsin and cleaned with an enzymatic cleaner (Tergazyme, Alconox, Inc., NY, USA). Frequency was measured again to get resonant frequency without cells only at the growth medium. Frequency drop was 2.92 KHz in the presence of cell. From these two frequencies (with and

without cells) single cell mass was calculated using Equation 5. Single HeLa cell mass was calculated as 1.01 ng, which is approximately half of the theoretical measurement of single HeLa cell mass i.e. 2.48 ng [10]. The experimented result was only 40% close to theoretical result and single cell mass result is to be fluctuated, depending on the numbers of cells attached on the cantilever. On the other hand the cell growth at the artificial cell cultivation media was not as normal as expected. LCA was to culture a single cell and measure the single cell mass only. But in dielectrophoresis, many cells were attached and frequency was measured with many cells acquainted in the cantilever [50]. In addition, the spring constant and the quality factor of the resonating cantilever is affected due to the small aspect ratio of the cantilever [51]. This may cause error to the calculating of spring constant of the cantilever. As a result, we could conclude that, LCA may require improvement in terms of cells capturing and spring constant calculation.

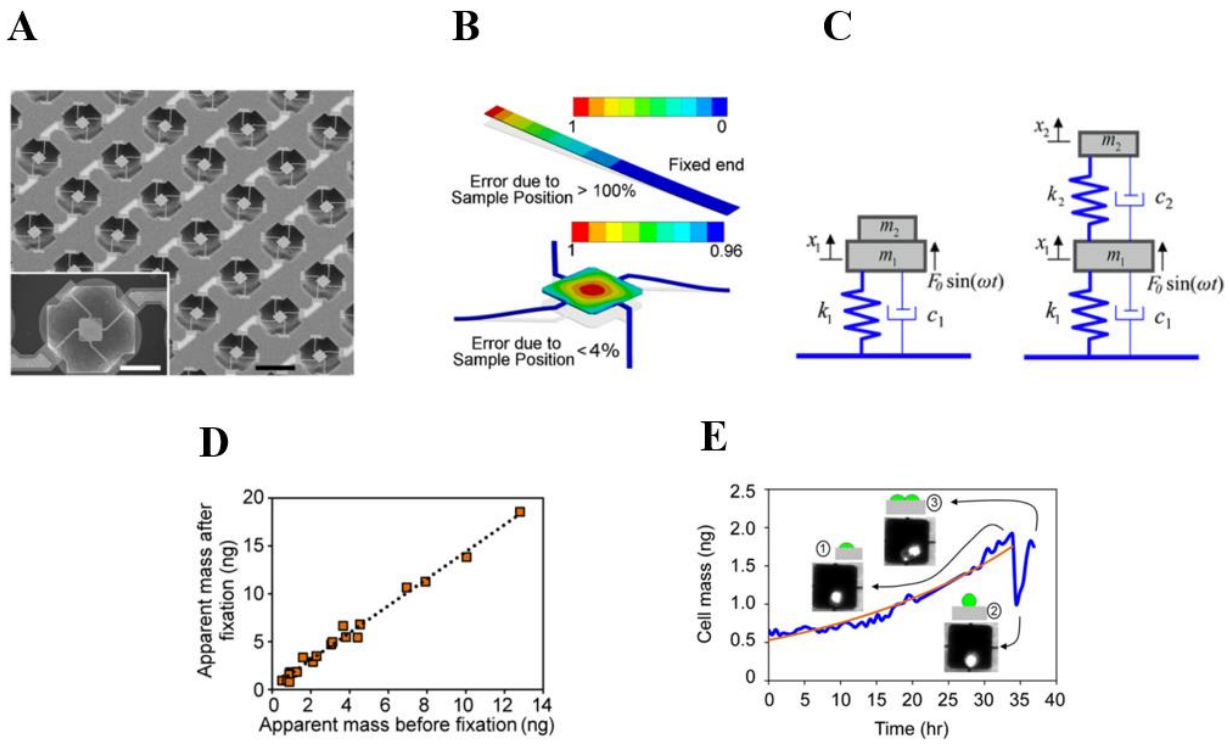



**Figure 7** Fabricated pedestal mass measurement sensor's arrays. There were 9×9=81 sensors fabricated. (B) For a typical cantilever sensor, error could be up 100% depending on the object's position, while for pedestal sensor the sensing error is less 4%. (C) Left is the linear mass-spring-damper model that have used for cantilever sensor, Right is the dynamic mass-spring-damper model for four beam pedestal mass measurement sensor. (D) Relation between fixed cell's apparent mass to the non-fixed corresponding cell's apparent mass. (E) Exponential increase of cell mass prior to cell division. Inset (1-3) showed the numerical model that used to represent cell division [16]

However, LOC resonating based cantilever mass measurement sensor enabled the technology to measure adherent cell mass directly. Major difference between SMR and 'living cantilever array' is that SMR generates the pick from the mass of the single cell. On the other hand, cantilever arrays shift the existed resonance depending on the mass of attached cells on the cantilever surface. Thermal noise was used to generate the vibration on the cantilever in LCA while electrostatic actuation for SMR.

## ■4.0 LAB-ON-CHIP PEDESTAL MASS MEASUREMENT SENSOR (PMMS)

Integrated lab-on-chip living cantilever arrays (LCA) considered as one of the successful work for measuring adherent cell mass. However, the cantilevers based mass sensor has non-uniform mass sensitivity [31], as a result accuracy depends on the cell position merely [10]. These issues have been addressed by object position independent pedestal mass measurement [16]. Furthermore, pedestal measurement sensor has an excellent geometrical shape that enables cell to be trapped within the mass sensing region. Previously, SMR was modified by introducing mechanical trap to the sensor to measure the buoyant mass at different liquids [30], but it was limited to non-adherent cell only. On the other hand, pedestal mass sensor was developed to measure the live adherent cell mass upon trapping and culturing on the pedestal surface.

There were 9x9 arrays where 81 pedestal (60 x 60 µm2) sensors were fabricated on the MEMS chip where each of the pedestal sensor was supported by four identical spring beam. Length of the supported beam was 80 µm, width was 40 µm with an approximate pit depth 50 µm. The entire fabrication process have been discussed elsewhere in [16]. Figure 7A shows the fabricated pedestal mass measurement sensor. The four beamed novel structure of the sensor generates the vertical vibration only and this structure reduces the amplitude fluctuation significantly [16]. This concept has been calibrated using numerical approaches. Results showed that the vibration direction is only vertically and the error of the mass sensitivity is less than 4% while it can be up to 100% for a conventional resonating cantilever depending on the position of the object on the cantilever [Figure 7B].

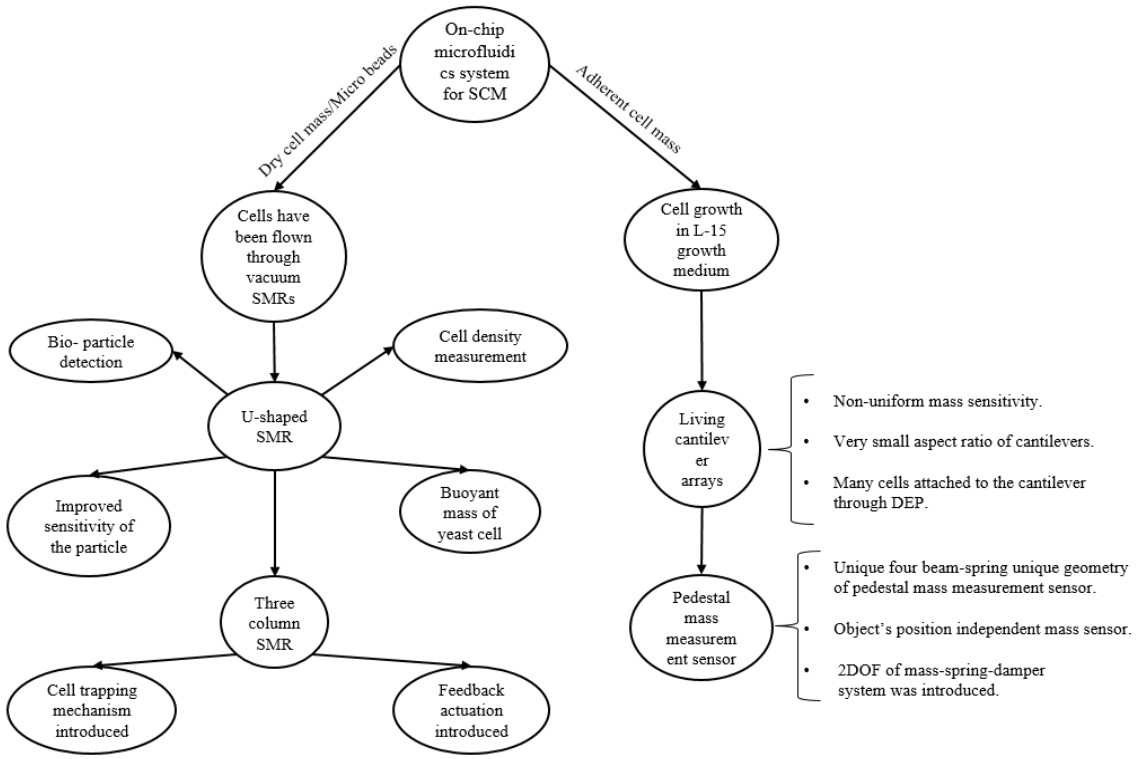



**Figure 8** Tree diagram to illustrate the technological advancements of single cell mass measurement. Left part indicating the resonator based mass sensors that have been used to measure non adherent and dry cell mass. Right hand indicating technological advancements that used to measure adherent live cell's mass

### 4.1 Procedures of the Single Cell Mass Measurement Using PMMS

Frequency shifting phenomenon was used to measure the cell mass using pedestal mass measurement sensor. Pedestal sensor was vibrated using Lorentz force by passing constant current of 150 μA in a static magnetic field. Three different frequencies of the resonator were measured. In air medium sensor was vibrated at the frequency of 150 KHz which had been used to calculate the spring constant of the supporting beam followed by measuring resonant frequency in L-15 (Sigma Aldrich) growth medium (60 KHz). Frequency measured inside the growth medium had been used a reference frequency. Human colon adenocarcinoma cell (HT29) was then cultured on the sensor to enable direct mass measurement. The sensor merged in the growth medium had been covered with PDMS hermetically with a covered slip. Finally, frequency of the pedestal platform had been measured in presence of live adherent cells, frequency was measured in every 30 min for 60 h to understand the relation between cell mass growth as well as the effect of cell migration through the sensor.

### 4.2 Relation Between Cell Mass, Stiffness and Growth

The conventional mass sensing cantilever was modelled based on linear mass-spring-damper system [16] [Figure 7C left]. This concept is valid when a cell has been fixed on the surface. But in liquid medium cells remain suspended and the vibration frequency may differ from the resonating cantilever which generates error to the frequency measurements. In the pedestal mass sensor dynamic mass-spring-damper has been proposed with 2 degree of freedom (DOF) to the beam spring which elucidates the effect of viscoelastic modulus to the mass sensor. Figure 7C (right) illustrate the dynamic mass-spring-damper model to improve mass measurement sensitivity. On the other hand, vibrating cell on the sensor have a finite elasticity as a result there might be variation in the vibration between cells and cantilever which leads the mass measurement results depend on cell stiffness as well. Using pedestal sensor apparent cell mass was measured for both attached and non-attached cells. For paraformaldehyde (PFA) cells apparent mass is 1.4 times higher for the attached cell than its corresponding non-attached cell on the same pedestal surface. This is because the cell stiffness increases for the fixed cells [16]. Figure 7D shows the results of measured mass for PFA fixed and non-fixed cells.

It is well known that single cell growth, division and death are a continuous process [13], [32], [52]. Previously, it was claimed that cell mass increases linearly with the growth through the entire cell cycle [34]. Later on it was proved that single cell mass merely depend on the cytoplasm of the cell which consists of enzymes, ribosome and other soluble components including water [32]. The growth of the cytoplasm is exponential [32] which generates the cell mass exponentially also known as cytoplasmic mass increase. On the other hand, the DNA replication of the single cell is also exponential and only few extraordinary case where DNA replication was linear [34]. But this extraordinary case is not adequate enough to support the linear replication of DNA [32], [53]. Pedestal mass measurement had developed the relationship between single cell mass and growth in prior to cell division. Figure 7E illustrated the exponential ($y = 0.5303e^{0353x}$) curve fitting of cell mass vs. growth for a particular cell which is in agreement with the previous arguments [16], [32].

However, directly measured SCM techniques enable the real time analysis of single cell mass at different phases of cell growth.

Micromechanical resonator integrated with microfluidic chip provides an excellent opportunity to extract a particular cell mass. Figure 8 describes the entire technological advancements of cell mass measurement techniques. We could say, this technological developments are continuously improved by the researcher. For example SMR was proposed to detect bio particles, optimized and applied to single cell mass measurement techniques. On the other hand, living cantilever arrays was proposed to measure mass of HeLa cells at fixed and non-fixed conditions. But the major drawback of the cantilever mass measurement sensor is the non-uniform mass sensitivity through the cantilever surface. This issue have been overcome by proposing four spring beam pedestal mass measurement system. Mass sensitivity of the pedestal mass measurement system is very promising and allow the sensor to measure mass for both adherent and non-adherent cells [16].

## ■5.0 CONCLUSION

In this work, we analysed and summarized the available methods of single cell mass measurement (SCM) from various published works. Although it is very challenging to develop a benchmark for SCM techniques, a detailed discussion of up-to-date microfluidics based lab-on-chip and mass spectrometry for SCM measurements techniques are presented throughout the entire review. For example lab-on-chip microfluidics system integrated with suspended micro channel resonator (SMR) for non-adherent cell mass measurement, 'living cantilever arrays' (LCA) for adherent cell measurement and also the position independent pedestal mass measurement sensor (PMMS) for measuring single cell mass directly. Comprehensive discussions of the relevant works including the pros and cons, mechanism, sensor geometry, fabrication procedures and the governing equations have been presented. Moreover, we tried to extract the key features from the relevant published works and reflect the accumulated information in this work. It is envisaged that, this article could be a one stop source for single cell mass analysers and could be a valuable direction for the future works in this area.

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## References

[1] M. R. Ahmad, M. Nakajima, S. Kojima, M. Homma, and T. Fukuda. 2010. Nanoindentation Methods to Measure Viscoelastic Properties of Single Cells Using Sharp, Flat, and Buckling Tips Inside ESEM. *IEEE Trans. Nanobioscience*. 9(1): 12–23.

[2] M. R. Ahmad, M. Nakajima, S. Kojima, M. Homma, and T. Fukuda. 2008. The effects of Cell Sizes, Environmental Conditions, and Growth Phases on the Strength of Individual W303 Yeast Cells Inside ESEM. *IEEE Trans. Nanobioscience*. 7(3): 185–93.

[3] M. R. Ahmad, M. Nakajima, S. Kojima, M. Homma, and T. Fukuda. 2011. Buckling Nanoneedle for Characterizing Single Cells Mechanics Inside Environmental SEM. *IEEE Trans. Nanotechnol*. 10(2): 226–236.

[4] M. R. Ahmad, M. Nakajima, S. Kojima, M. Homma, and T. Fukuda. 2008. In Situ Single Cell Mechanics Characterization of Yeast Cells Using Nanoneedles Inside Environmental SEM. *IEEE Trans. Nanotechnol*. 7(5): 607–616.

[5] I. Obataya, C. Nakamura, S. Han, N. Nakamura, and J. Miyake. 2005. Nanoscale Operation of a Living Cell Using An Atomic Force Microscope with a Nanoneedle. *Nano Lett.* 5(1): 27–30.

[6] R. J. Fasching, S. J. Bai, T. Fabian, and F. B. Prinz. 2006. Nanoscale Electrochemical Probes for Single Cell Analysis. *Microelectron. Eng.* 83, 4–9, 1638–1641.

[7] T. P. Burg, M. Godin, S. M. Knudsen, W. Shen, G. Carlson, J. S. Foster, K. Babcock, and S. R. Manalis. 2007. Weighing of Biomolecules, Single Cells and Single Nanoparticles in Fluid. *Nature*. 446(7139): 1066–1069.

[8] S. E. Cross, Y.-S. Jin, J. Rao, and J. K. Gimzewski. 2007. Nanomechanical Analysis of Cells from Cancer Patients. *Nat. Nanotechnol.* 2(12): 780–783.

[9] M. Godin, A. K. Bryan, T. P. Burg, K. Babcock, and S. R. Manalis. 2007. Measuring the Mass, Density, and Size of Particles and Cells Using a Suspended Microchannel Resonator. *Appl. Phys. Lett.* 91(12): 123121–1231212.

[10] K. Park, J. Jang, D. Irimia, J. Sturgis, and J. Lee. 2008. Living Cantilever Arrays' for Characterization of Mass of Single Live Cells In Fluids. *Lab Chip*. 8: 1034–1041.

[11] F. Xia, W. Jin, X. Yin, and Z. Fang. 2005. Single-cell Analysis by Electrochemical Detection with a Microfluidic Device. *J. Chromatogr. A*. 1063(1–2): 227–233.

[12] I. Kubo, S. Furutani, and K. Matoba. 2011. Use of a Novel Microfluidic Disk in the Analysis of Single-cell Viability and the Application to Jurkat Cells. *J. Biosci. Bioeng.* 112(1): 98–101.

[13] A. K. Bryan, A. Goranov, A. Amon, and S. R. Manalis. 2010. Measurement of Mass, Density, and Volume During the Cell Cycle of Yeast. *Proc. Natl. Acad. Sci*. 107(3): 999–1004.

[14] F. S. O. Fritzsch, C. Dusny, O. Frick, and A. Schmid. 2012. Single-cell Analysis in Biotechnology, Systems Biology, and Biocatalysis. *Annu. Rev. Chem. Biomol. Eng.* 3: 129–55.

[15] J. Lee, R. Chunara, W. Shen, K. Payer, K. Babcock, T. P. Burg, and S. R. Manalis. 2011. Suspended Microchannel Resonators with Piezoresistive Sensors. *Lab Chip*. 11(4): 645–51.

[16] K. Park, L. J. Millet, N. Kim, H. Li, X. Jin, G. Popescu, N. R. Aluru, K. J. Hsia, and R. Bashir. 2010. Measurement of Adherent Cell Mass and Growth. *Proc. Natl. Acad. Sci*. 107(48): 20691–96.

[17] M. Y. M. Chiang, Y. Yangben, N. J. Lin, J. L. Zhong, and L. Yang . 2013. Relationships among cell morphology, intrinsic cell stiffness and cell-substrate interactions. *Biomaterials*. 34(38): 9754–62.

[18] G. Weder, M. C. Hendriks-Balk, R. Smajda, D. Rimoldi, M. Liley, H. Heinzelmann, A. Meister, and A. Mariotti. 2014. Increased Plasticity of the Stiffness of Melanoma Cells Correlates with Their Acquisition of Metastatic Properties. *Nanomedicine*. 10(1): 141–8.

[19] M. H. Rahman, A. H. Sulaiman, M. R. Ahmad, and T. Fukuda. 2013. Finite Element Analysis of Single Cell Wall Cutting by Piezoelectric-Actuated Vibrating Rigid Nanoneedle. *IEEE Trans. Nanotechnol.* 12(6): 1158–1168.

[20] S. Suresh. 2007. Biomechanics and Biophysics of Cancer Cells. *Acta Biomater*. 3(4): 413–38.

[21] A. Schmid, H. Kortmann, P. S. Dittrich, and L. M. Blank. 2010. Chemical and Biological Single Cell Analysis. *Curr. Opin. Biotechnol.* 21(1): 12–20.

[22] D. Di Carlo and L. P. Lee. 2006. Dynamic Single-cell Analysis for Quantitative Biology. *Anal. Chem.* 78(23): 7918–7925.

[23] J. Nilsson, M. Evander, B. Hammarström, and T. Laurell. 2009. Review of Cell and Particle Trapping in Microfluidic Systems. *Anal. Chim. Acta*. 649(2): 141–57.

[24] B. Ilic, H. G. Graighead, S. Krylov, W. Senaratne, and P. Neuzil. 2004. Attogram Detection Using Nanoelectromechanical Oscillators. *J. Appl. Phys.* 95(7): 3694–3703.

[25] Y. T. Yang, C. Callegari, X. L. Feng, K. L. Ekinci, and M. L. Roukes. 2006. Zeptogram-scale Nanomechanical Mass Sensing. *Nano Lett.* 6(4): 583–86.

[26] A. Gupta, D. Akin, and R. Bashir. 2004. Single Virus Particle Mass Detection Using Microresonators with Nanoscale Thickness. *Appl. Phys. Lett.* 84(11): 1976–1978.

[27] D. Lange, C. Hagleitner, A. Hierlemann, O. Brand, and H. Baltes. 2002. Cantilever Arrays on a Single Chip: Mass-sensitive Detection of Volatile Organic Compounds. *Anal. Chem.* 74(13): 3084–3095.

[28] B. Ilic, Y. Yang, and H. G. Craighead. 2004. Virus detection using nanoelectromechanical devices. *Appl. Phys. Lett.* 85(13): 2604–06.

[29] T. P. Burg and S. R. Manalis. 2003. Suspended Microchannel Resonators for Biomolecular Detection. *Appl. Phys. Lett.* 83(13): 2698–2700.

[30] Y. Weng, F. F. Delgado, S. Son, T. P. Burg, S. C. Wasserman, and S. R. Manalis. 2011. Mass Sensors with Mechanical Traps for Weighing Single Cells in Different Fluids. *Lab Chip*. 11(24): 4174–80.

[31] S. Dohn, R. Sandberg, W. Svendsen, and A. Boisen. 2005. Enhanced Functionality of Cantilever Based Mass Sensors Using Higher Modes. *Appl. Phys. Lett*. 86(23): 233501–03.

[32] S. Cooper. 2006. Distinguishing between Linear and Exponential Cell Growth During the Division Cycle: Single-Cell Studies, Cell-Culture Studies, and the Object of Cell-Cycle Research. *Theor. Biol. Med. Model.* 3: 10–25.
[33] S. De Flora, A. Izzotti, K. Randerath, E. Randerath, H. Bartsch, J. Nair, R. Balansky, F. Schooten, P. Degan, G. Fronza, D. Walsh, and J. Lewtas. 1996. DNA Adducts and Chronic Degenerative Diseases. Pathogenetic Relevance and Implications in Preventive Medicine. *Mutat. Res. Genet. Toxicol.* 366(3): 197–238.
[34] J. M. Mitchison. 2005. Single Cell Studies of the Cell Cycle and Some Models. *Theor. Biol. Med. Model.* 2: 4–9.
[35] C. J. Lord and A. Ashworth. 2012. The DNA Damage Response and Cancer Therapy. *Nature*. 481(7381): 287–94.
[36] C. Fenselau and P. A. Demirev. 2002. Characterization of Intact Microorganisms by MALDI Mass Spectrometry. *Mass Spectrom. Rev.* 20(4): 157–71.
[37] D. Sarid. 1994. *Scanning Force Microscopy : with Applications to Electric, Magnetic, and Atomic Forces*. Oxford University Press, USA.
[38] W. H. Grover, A. K. Bryan, M. Diez-Silva, S. Suresh, J. M. Higgins, and S. R. Manalis. 2011. Measuring Single-cell Density. *Proc. Natl. Acad. Sci.* 108(27): 10992–6.
[39] J. L. Arlett and M. L. Roukes. 2010. Ultimate and Practical Limits of Fluid-based Mass Detection with Suspended Microchannel Resonators. *J. Appl. Phys.* 108(8): 084701–11.
[40] J. Lee, A. K. Bryan, and S. R. Manalis. 2011. High Precision Particle Mass Sensing Using Microchannel Resonators In The Second Vibration Mode. *Rev. Sci. Instrum.* 82(2): 02370401–04.
[41] J. Bühler, F. P. Steiner, and H. Baltes. 1997. Silicon Dioxide Sacrificial Layer Etching in Surface Micromachining. *J. Micromechanics Microengineering*. 7(1): 1–13.
[42] J. W. Berenschot, N. R. Tas, T. S. J. Lammerink, M. Elwenspoek, and A. Berg. 2002. Advanced Sacrificial Poly-Si Technology for Fluidic Systems. *J. Micromechanics Microengineering*. 12(5): 621–624.
[43] E. B. Cummings and A. K. Singh. 2003. Dielectrophoresis in Microchips Containing Arrays of Insulating Posts: Theoretical and Experimental Results. *Anal. Chem.* 75(18): 4724–31.
[44] D. Di Carlo, N. Aghdam, and L. P. Lee. 2006. Single-cell Enzyme Concentrations, Kinetics, and Inhibition Analysis Using High-density Hydrodynamic Cell Isolation Arrays. *Anal. Chem.* 78(14): 4925–30.
[45] J. E. Molloy and M. J. Padgett. 2002. Lights, Action: Optical Tweezers. *Contemp. Phys.* 43(4): 241–258.
[46] M. Evander, L. Johansson, T. Lilliehorn, J. Piskur, M. Lindvall, S. Johansson, M. Almqvist, T. Laurell, and J. Nilsson. 2007. Noninvasive Acoustic Cell Trapping in a Microfluidic Perfusion System for Online Bioassays. *Anal. Chem.* 79(7): 2984–91.
[47] F. Chowdhury, S. Na, D. Li, Y. C. Poh, T. S. Tanaka, F. Wang, and N. Wang. 2010. Material Properties of the Cell Dictate Stress-induced Spreading and Differentiation in Embryonic Stem Cells. *Nat. Mater.* 9(1): 82–88.
[48] A. J. Engler, S. Sen, H. L. Sweeney, and D. E. Discher. 2006. Matrix Elasticity Directs Stem Cell Lineage Specification. *Cell*. 126(4): 677–89.
[49] A. Gupta, D. Akin, and R. Bashir. 2004. Single Virus Particle Mass Detection Using Microresonators with Nanoscale Thickness. *Appl. Phys. Lett.* 84(11): 1976–1978.
[50] M. S. Pommer, Y. Zhang, N. Keerthi, D. Chen, J. a Thomson, C. D. Meinhart, and H. T. Soh. 2008. Dielectrophoretic Separation of Platelets from Diluted Whole Blood in Microfluidic Channels. *Electrophoresis*. 29(6): 1213–8.
[51] J. E. Sader, J. W. M. Chon, and P. Mulvaney. 1999. Calibration of Rectangular Atomic Force Microscope Cantilevers. *Rev. Sci. Instrum.* 70(10): 3967–3969.
[52] D. Di Carlo, L. Y. Wu, and L. P. Lee. 2006. Dynamic Single Cell Culture Array. *Lab Chip*. 6(11): 1445–9.
[53] J. M. Mitchison. 2003. Growth During the Cell Cycle. *Int. Rev. Cytol.* 226: 165–258.