Lab on a Chip

FOCUS

New technologies for measuring single cell mass

Gabriel Popescu,*[abcd] Kidong Park,†[abe] Mustafa Mir†[acf] and Rashid Bashir*[abcd]

Cite this: *Lab Chip*, 2014, **14**, 646

Received 9th September 2013,
Accepted 19th November 2013

DOI: 10.1039/c3lc51033f

www.rsc.org/loc

Characterizing the physical properties of single cells is of great interest for unraveling the heterogeneity in a cell population. Recent advances in micro-systems technologies and quantitative imaging have enabled measurements of the mass and growth of single cells. So far, three classes of techniques have been reported for such measurements. These are suspended micro-channel resonators (SMR) sensors, quantitative phase imaging (QPI), and pedestal resonant sensors. Here we compare the unique merits of each method and discuss their potential to evolve into multi-modal platforms for answering fundamental questions in biology and medicine.

Introduction

It has become increasingly evident that there is a strong interplay between the molecular scale chemical interactions within a live cell, and the cell's physical behavior at the meso-scale. The ability to precisely measure the physical properties of these single cells such as their mass, density, or stiffness has only become possible due to the recent development of new measurement techniques. These developments have allowed the exploration of important biological phenomena. For example, the very basic question of whether heavier cells grow faster (suggesting an exponential growth pattern) or whether the growth rate is independent of cell size (indicating linear growth) has been extremely difficult to answer. If the growth rate is constant with cell size, perhaps cells do not need a sophisticated machinery to maintain size homeostasis; conversely, an increase in growth rate with cell size requires checkpoints and regulatory systems to maintain a constant size distribution as explained below. Besides its importance from a basic science point of view, understanding cell growth in the context of various chemo-mechanical stimuli can enable the development of new drugs capable of modulating growth in a predictable fashion and impact the understanding and treatment of many diseases.

Until the early 2000's, most reports on cell growth had primarily focused on "simpler" cell types, such as yeast and bacterial cells.[1,2] These studies were conducted using impedance counters or simple morphological measurements using microscopy. In yeast cells, an exponential growth pattern was measured and it was shown that yeast cells continue to grow even if cell cycle progression is blocked indicating that a size check point must exist.[3] When media conditions were switched, the yeast cells were able to adjust their size prior to dividing. These studies led to the conclusion that a size check point must exist and thus cells should have a mechanism for measuring their own size. It was believed that mammalian cells must operate on a similar principle,[3] since growth control mechanisms must be fundamental and thus should be conserved across different cell types.

In 2003, Conlon and Raff reported measurements on the size distributions (using an impedance counter) of proliferating primary rat Schwann cells, while blocking cell division, and under the influence of various growth factors.[4,5] Their data showed that the cells undergo linear growth when they aren't allowed to divide and that the net rate of protein production is independent of cell size. When the media nutrient conditions were changed, unlike the yeast cells, rat cells took several cycles to adjust their size. The authors concluded that since metazoan cells are less individualistic than yeast, the differences in the animal cell growth control must be due to the heavy dependence on extracellular signals and less selective pressure to respond to environmental conditions.[5] It follows that if the growth is regulated by extra-cellular signals, then mammalian cells do not require a sizing mechanism or checkpoint. It was suggested that if cell growth is exponential then a cell sizing mechanism must exist, otherwise variability in the division size would increase with each generation. On the other hand if the growth is linear, such a

[a] *Department of Electrical and Computer Engineering, University of Illinois at Urbana-Champaign, Urbana, IL, 61821, USA. E-mail: gpopescu@illinois.edu, rbashir@illinois.edu*
[b] *Micro and Nanotechnology Laboratory, University of Illinois at Urbana-Champaign, Urbana, IL, 61821, USA*
[c] *Beckman Institute of Advanced Science and Technology, University of Illinois at Urbana-Champaign, Urbana, IL, 61821, USA*
[d] *Department of Bioengineering, University of Illinois at Urbana-Champaign, Urbana, IL, 61821, USA*
[e] *Division of Electrical and Computer Engineering, Louisiana State University, Baton Rouge, LA, 70803, USA*
[f] *California Institute of Quantitative Biosciences, University of California Berkeley, Berkeley, CA, 94707, USA*
† Authors contributed equally.

sizing mechanism would not be necessary and cells could rely on extra-cellular signals or growth rate measurements to regulate their size.[3,4,6] This interpretation has been disputed by Cooper who argues that theoretically, a size checkpoint would be equally effective in both the linear and exponential cases.[7,8] This can be understood by considering that, if newborn cells have variable growth rates (as in an exponential model), and if the cells still divide at the same size but at varying times, then homeostasis can still be maintained.

Experimentally, differentiating these two growth trends is extremely challenging since a 6% or better resolution in cell size is required.[9] To study growth in context of the cell cycle, the population must be synchronized, a technique which offers poor resolution and is known to introduce artifacts such as oversized cells.[4,7] In 1962, Collins and Richmond proposed a way to calculate growth rate from measuring size distributions of asynchronous populations.[10] However, it was difficult to apply this method since it requires a precise measurement of the size differences of newborn daughter cells. Tzur *et al.*[9] overcame this hurdle by developing a method in which only one daughter cell remains attached to a membrane upon division; the second is released and measured. They found that growth in lymphoblasts is exponential soon after division and during most of the cell cycle. They found that larger and older cells tend to divide earlier indicating that both a size and time checkpoint may exist. Mechanisms for such size and age checkpoints have not yet been identified for "higher" eukaryotes.

Direct and accurate characterization of growth rate can also deepen our understanding on a variety of pathological conditions of cells. For instance, one of the hallmarks of cancer is unregulated growth. In most tumors, the PI3K/AKT/mTOR pathway, which is major pathway to regulate the growth rate, is up-regulated either by activation of upstream regulators such as RAS or by the loss of negative regulators such as PTEN.[11] For this reason, mTOR is actively investigated as a target pathway for cancer therapy[12] and understanding the implication of mTOR signaling on the cell growth would elucidate fundamental mechanism of cancer development. Besides, abnormal control of cell growth can lead to cardiac hypertrophy and developmental disorders such as tuberous sclerosis complex, Peutz–Jeghers syndrome,[13] and Lhermitte–Duclos disease.[11]

Despite much effort, the basic question of how cell growth is regulated across the cell cycle and the changes of its microenvironment is still under debate. It has become increasingly clear that to truly understand mammalian cell growth, measurements must be performed at the single cell level with high precision and high throughput.[14] Furthermore, while cell volume (as measured by impedance counters) may be used as a surrogate for cell size, it has been recognized that the measurement of cell mass more accurately reflects the accumulation of cellular contents. Therefore in order to accurately measure cell size any measurement platform must fulfill the following basic criteria: (i) as a typical live cell's mass is of the order of 100–1000 pg, the method should allow for a mass sensitivity of 1% or less (~1 pg or less). (ii) The typical cell cycle is on the order of 20 hours, during which the cell doubles its mass; thus the measurements should be able to measure the growth rate of a same cell for at least over 20 hours and preferably over a few days. (iii) Typically, the cell undergoes drastic morphological changes during the cell cycle (*e.g.*, rounding up and reducing the cell contact area for adherent cells during mitosis); therefore, the measurement should decouple mass information from morphology. (iv) The throughput of the measurement should be high enough to achieve statistical reliability and characterize cell-to-cell variations. The required throughput is highly dependent on the application and the natural heterogeneity in the population being measured. Given a measured population mean value and standard deviation, conventional procedures for determining the correct sample size should be used.

In addition to these basic criteria an ideal technology will also have the following capabilities: (i) since it is known that cell–cell interactions modulate the cell growth, the ideal method should operate equally well on single cells and, in parallel, on confluent populations. (ii) Most biochemical assays rely on fluorescence measurements and hence the method should be compatible with fluorescence microscopy to allow simultaneous measurement of cell growth and biochemical status.

The approaches proposed so far for fulfilling these requirements can be differentiated into *optical* and *mechanical* methods. Three methods have been developed to measure the 'mass' of an individual living cell. These are (i) optically-based measurements of the 'dry mass' of adherent cells,[15–20] (ii) suspended micro-channel resonator (SMR) based measurements of the 'buoyant mass' of suspended cells flowing through a U-shaped micro-channel integrated in a cantilever,[21–25] and (iii) pedestal resonant sensor based measurements of the 'total mass' of adherent cells.[26,27]

Confocal microscopy[28] and other emerging imaging techniques on cells with GFP-tagged structural proteins, can also be an alternative approach to estimate the growth of a single cell. Confocal imaging is widely used to directly measure cell volume, which is equivalent to the cell mass assuming a constant density. Furthermore advanced imaging techniques, such as PALM and STED,[29] can also be used to measure the volume of cells. However these methods require elaborate sample preparation (*i.e.* transfection or staining) for live imaging and are limited in throughput. Thus, they are not covered in this article. In the remainder of this perspective we will discuss how the new optical and mechanical approaches work, a comparison of their capabilities, the new insights they have provided on cell growth, and how they may be applied and improved in the future.

Quantitative Phase Imaging (QPI)

Optical interferometric measurements of growth rely on the intrinsic relationship between the refractive index of cells and the non-aqueous content (such as proteins, nucleic acids, and lipid molecules) of the cells (Fig. 1). The measurable optical

path length shift introduced by a cell to transmitted light is proportional to the cell's mass density. In order to optically measure a density map of a single cell, one must *quantitatively* acquire the optical path length map associated with the cell.[17] In the past decade, various methods have been developed and *quantitative phase imaging* has become a rapidly growing field both in technology development and novel applications in biomedicine.[18] Spatial light interference microscopy (SLIM)[19] is a recently developed quantitative phase imaging method that provides highly sensitive measurements of optical path-length, corresponding to dry mass sensitivities on the order of 0.1–1 fg μm^{-2}. SLIM can noninvasively measure the growth of single cells and populations over time scales spanning from milliseconds to weeks.[19] Importantly, SLIM yields dry mass maps that innately overlay with the optical imaging including fluorescence. Therefore, cell growth rate, cell morphology, and fluorescent markers can be characterized simultaneously.

Using SLIM, exponential growth in *E. coli* growth was measured and from morphological measurements it was determined that the density of these cells remains constant throughout their life cycle.[19] Using a fluorescent reporter for the S phase of the cell cycle, eukaryotic single cell growth was measured over each phase of the cell cycle and found that the G2 phase exhibits the highest growth rate, which is mass dependent. This study marked the first direct measurement of cell cycle dependent growth at the single cell level. Since SLIM can simultaneously be used to measure motility and cellular dynamics it was used to study how cell adherence to the substrate modulates growth in cell clusters. It was found that adherence enforced by poly-L-lysine tends to lower growth rates.[20]

In addition to measuring single cell growth, cell age, morphology and motility, SLIM simultaneously provides mass and spatial organization information at the population level. This enables studying how the emergent behavior of the culture arises from cellular level phenomenon and how cell–cell interactions modulate this behavior. Interestingly, QPI can also render dynamic dry mass density maps, which in turn provides information about intracellular mass transport.[30,31] So far this technique has been successfully applied to study growth in human osteosarcoma and breast cancer cells, bacteria, drosophila embryonic cells and neurons and was found to be compatible with all these cell types.[32]

Suspended Micro-channel Resonator (SMR)

A resonant frequency of a MEMS resonant mass sensor decreases with an increase in the total mass of the sensor. A MEMS resonant mass sensor measures the mass from the difference of the resonant frequencies with and without a loaded sample. MEMS resonant mass sensors have been widely used to measure the mass of biologically important entities, such as DNA, viruses, bacteria, and fungi. However, the quality factor of the mechanical resonance and the mass sensitivity of the MEMS mass sensors are severely compromised in liquid and thus these studies measured the mass of dried samples in air or in vacuum. For this reason, it has been a challenging task to characterize the growth rate of mammalian cells using MEMS mass sensors.

To achieve a high mass resolution in 'wet samples', the SMR adopted a unique structure incorporating a microfluidic channel integrated inside a suspended micro-cantilever in vacuum (Fig. 2). In this approach, sample particles suspended in a biological media flow through the microfluidic channel and the total mass of the cantilever increases when a particle is passing through the microfluidic channel inside the cantilever. This temporal increase of the total mass reduces the resonant frequency of the cantilever, which is optically detected. Since the cantilever is in vacuum, SMR shows a very high quality factor[21–25] and mass resolution of 25–50 fg for 'wet samples'.[23] Since SMR senses the difference between the mass of the sample particle and the mass of the media replaced by the particle, it measures the 'buoyant mass' defined by $m_{buoyant} = V(\rho_{cell} - \rho_{media})$, where ρ is mass density and V is cell volume. The density of the water is similar to that of the media and the buoyant mass is equivalent to the amount of biomass in the cell, similar to the optically measured 'dry mass' described in the quantitative phase imaging earlier.

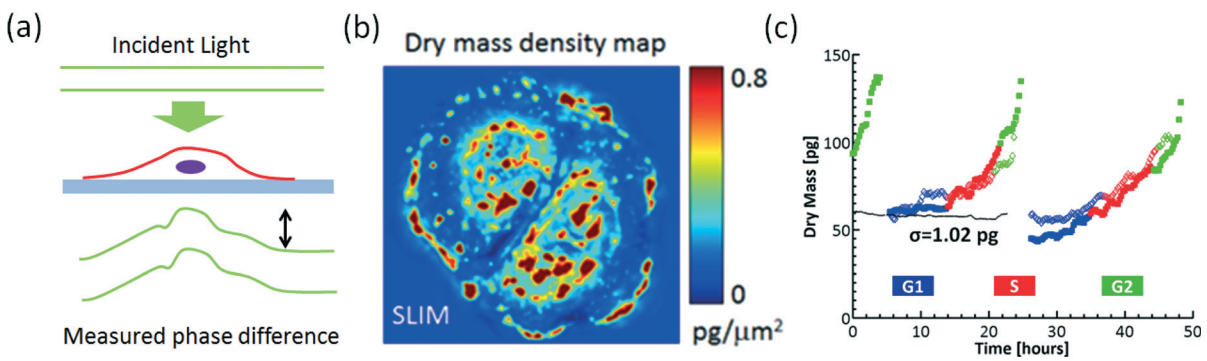

Fig. 1 Optical measurements of cell mass and growth. (a) In quantitative phase imaging the path length delay of light travelling through a cell is measured as a phase difference which is directly proportional to the dry mass density of the cell at each point in the image. (b) Dry mass density map. (c) The growth characteristics measured with PCNA cell cycle reporter (adapted and reproduced with permission from ref. 19).

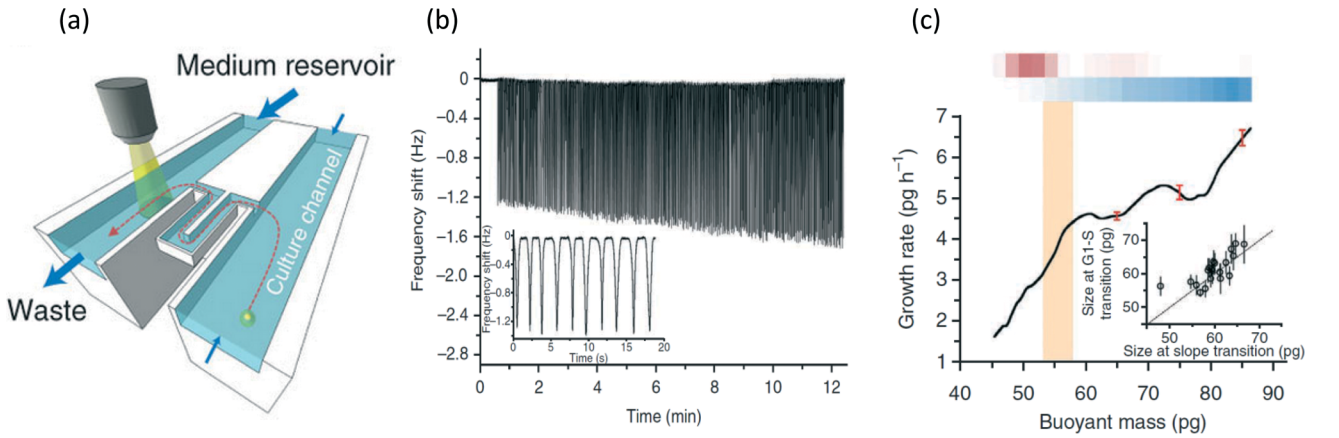

Fig. 2 Suspended Micro-channel Resonator (SMR). (a) Cell flows through a hollow cantilever filled with a liquid. The resonator is electrostatically driven and its resonant frequency is optically detected. (b) Changes in resonant frequency (and hence the buoyant mass) are detected *versus* time as the cell flows back and forth in the microchannel in the cantilever. Each drop in the frequency is a single passage of the cell though the microchannel. (c) In conjunction with mass measurements, the cell cycle was monitored with fluorescent cell cycle reporter, FUCCI (adapted and reproduced with permission from ref. 21, 25).

Originally, SMR had a flow-through configuration similar to flow cytometers,[22] but a series of technical innovations have transformed SMR to be suitable for growth characterization at a single cell level. Automated external fluid control enabled the mass measurement of the same cell over time by repeatedly moving the same cell back and forth.[21] Furthermore, an optically transparent window on the microfluidic channel allowed the use of fluorescent reporters for cell cycle determination.[25] Using these SMR devices, it has been found that for bacteria (*E. coli K12* and *B. subtilis*), yeast (*S. cerevisiae*) and mammalian cells (L1210 mouse), the growth rate increases with increasing mass.[21] By simultaneously measuring cell mass and cell cycle using the FUCCI reporter system, the SMR technology was used to determine that growth rate is a more complex function of cell mass. It was shown that growth rate increases in G1 with cell mass but decreases in the S phase and that the G1–S transition is correlated to a critical growth rate rather than the mass itself.[25] Most recently, SMR with a constriction inside the microfluidic channel has been developed to characterize cell's deformability and surface friction in addition to cell mass.[33]

Pedestal resonant sensor

Pedestal resonant sensors can measure the whole cell mass of adherent cells, including the fluid and the non-aqueous contents. It measures the mass of a cell attached to a silicon pedestal continuously over time in a position independent manner. The majority of MEMS resonant mass sensors were based on a cantilever structure for extreme miniaturization and higher mass sensitivity. However, these cantilever-based mass sensors have a non-uniform mass sensitivity, meaning that the mass output of the sensor depends on the location of the sample. Specifically, this non-uniform mass sensitivity interprets a cell migration as an increase or decrease of cell mass. To address this issue, the pedestal resonant sensor uses a structure consisting of a rectangular platform suspended by four folded springs, to maintain the spatial variation of the mass sensitivity within 4% in the platform[34,35] (Fig. 3). Furthermore, electromagnetic actuation and statistical algorithms were used to enhance the mass resolution in a fluid environment (a problem the SMR overcomes by encapsulating the cells and the liquid inside the micro-fabricated tube and having vacuum outside, thus resulting in much lower damping). However, due to the higher damping of the vibrating pedestal in fluid, pedestal resonant sensors have much lower sensitivity than SMR. Mass resolutions on the order of 1 pg have been reported.[26]

The pedestal mass sensors are also dependent on the mechanical coupling between the pedestal and the sample, including the sample's mechanical stiffness.[26] To address this dependency, this method models the cell and pedestal as two coupled oscillators, where the cell also has a finite stiffness and viscosity. The 'apparent mass' decreases when the cell's stiffness is below a certain level. Therefore, for soft cells, the knowledge of the cell stiffness is required to extract the total mass – which in turn could be used to obtain stiffness if the mass was known. From the mass measurement of the same cell before and after chemical fixation (which hardens cells), the stiffness of colon carcinoma cells (HT29) was extracted exploiting this dependency. Assuming a constant stiffness, it was found that the cells exhibited an exponential growth rate, *i.e.* the cells grew faster as they became larger. In addition, this method can detect the characteristic 'balling-up' of cells during the division especially for soft cells (less than ~25 kPa). During cell division, the cells undergo rapid changes in its morphology and stiffness, which reduces the mechanical coupling between the cell and the platform, resulting in a sharp decrease of the apparent mass. Compared to SMR and QPI, the pedestal resonant sensor has had limited reports after the first publication.[26]

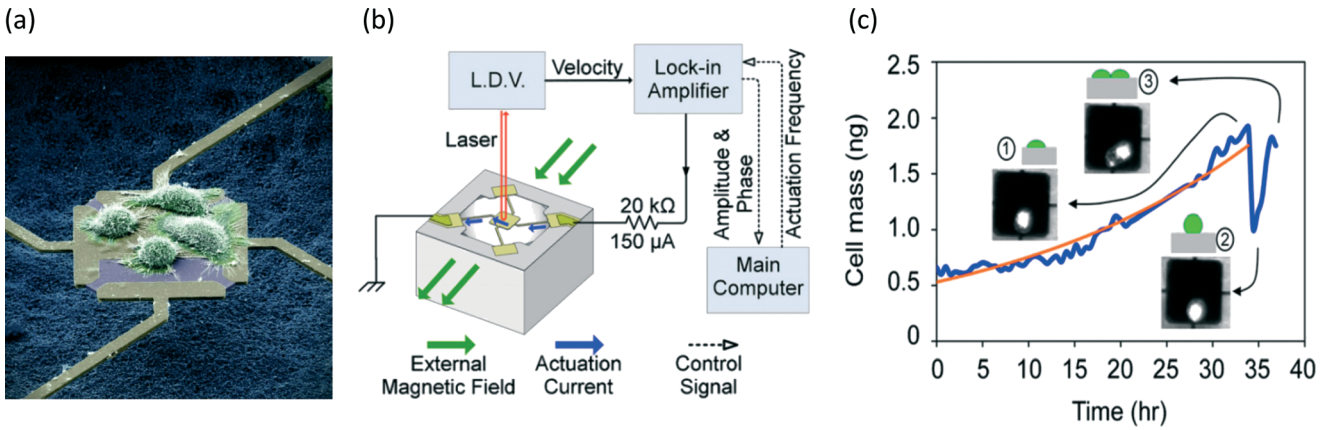

Fig. 3 Pedestal Resonant Sensor. (a) Cells are cultured on a platform for the mass measurements. (b) The phase of the velocity was measured with a laser Doppler vibrometer to extract the changes of resonant frequency and thus cell growth. (c) The change of the single cell mass was obtained with cell imaging. The cell mitosis event was reflected as a sharp decrease on the cell growth profile (adapted and reproduced with permission from ref. 26).

Discussion and outlook

Each technique described above has its own advantages and unique merits compared to the others (Table 1). It should be noted that in all the methods the actual mass growth curves appear to be noisier than the reported sensitivities. Such fluctuations are expected due to the stochastic nature of all biological systems and understanding their nature is of great interests. For mass sensitivity, SMR shows the highest mass resolution, which is 0.05% of total buoyant mass (or dry mass) of a single cell. In addition to the cell mass measurement, it can monitor fluorescent biomarkers and precisely measure the cell density using media of different mass densities. Although this technique has the potential for measuring adherent cells by trapping them in the microchannel, so far only measurements of suspended samples have been reported. SLIM can measure the dry mass of adherent and suspended cells along with high-resolution optical imaging including fluorescence microscopy. With image processing, SLIM can measure the growth rates of individual cells among confluent population with cell-to-cell contacts and achieve high measurement throughput. However, rapid morphological changes,

Table 1 Characteristics of the three methods for measurement of cell mass

Method characteristics	Spatial light interference microscopy	Suspended microchannel resonator	Pedestal resonant sensor
Comments	Measures 'dry mass' of live cells.	Measures 'buoyant mass' of live cells (same as SLIM based 'dry mass').	Measures total mass (dry+water) of cells. But measurement is coupled with stiffness and viscosity of cells.
1. Mass sensitivity (actual sensitivity varies due to presence of debris in the culture)	0.1% of dry cell mass.	0.05% of cell buoyant mass.	~1% of total cell mass.
	~50–100 fg for typical mammalian cells of 50–100 pg of dry mass.	~25 fg for typical mammalian cell of 50–100 pg of buoyant mass.	~10 pg for typical mammalian cell of 1 ng of total mass.
2. Measure cell mass *versus* time	Yes.	Yes. By flowing cells back and forth through sensor area.	Yes.
3. Sensitivity to subcellular mass distribution	Yes, ~0.1–1 fg μm^{-2}	No.	No.
4. Measure cell morphology	Yes.	Not reported yet.	Initial report of stiffness and mass.
5. Single cells in confluent cell populations	Yes, with image processing.	Single cells in suspension.	Single cells but not confluent cell populations.
6. Adherent *versus* non-adherent	Adherent cells reported. Also possible in a flow system.	Non-adherent cells reported. Suspended adherent cells reported for single-time-point measurement.	Adherent cells only.
7. Combination with fluorescence	Yes.	Yes.	Possible but not reported yet.

for example the rounding of cells during mitosis, can potentially affect the measurements unless proper precautions (sufficient sampling in the axial direction) are taken. SLIM measurements can also be affected by debris present in the culture media. To minimize this source of error the media can be changed between time points to remove dead cells and other impurities. Lastly, pedestal resonant sensors are applicable to the adherent cells and are sensitive to the mechanical coupling between cells and sensors, including cell stiffness and adhesion. To achieve a mass measurement with less dependency on the mechanical coupling, it is advisable to lower the resonant frequency of the pedestal sensor, at which the inertia of the cell mass can be sufficiently supported by the cell body. In addition, they can measure the whole cell mass and have the potential to measure the exchange of fluids and cellular contents across the cell membranes.

One of the practical challenges for measuring cell mass is maintaining appropriate environmental conditions during the measurement. With SMR, the suspended cell is cultured inside a microfluidic channel and exposed to shear stress which has recently been minimized by storing the cell in a large bypass reservoir and occasionally measuring its mass.[25] Nutrients are provided from the larger inlet/outlet channel and the bypass reservoir through diffusion and convection.[21] In the pedestal resonant sensor, the cell is cultured on the sensor surface immersed in a growth media contained by a micro-well of 100 μL volume. SLIM can be used with any chamber or microfluidic device that is optically transparent. Regarding the measurement interval, all of the three methods can have a high enough temporal resolution for cell growth measurements. SMR has demonstrated the minimum measurement interval, typically 30 seconds for the mass measurement and 30 minutes for fluorescence.[25] SLIM can measure the mass density map of a 390 × 290 μm^2 area (~4–5 cells) at a rate of 12.5 Hz,[36] however typically a 1.5 × 1.22 mm^2 area is scanned every 30 minutes. The pedestal resonant sensor typically measures the cell mass every 30 minutes, while it takes about 1 minute to measure the mass of a single cell. The number of cells that can be simultaneously measured is another important factor, affecting the measurement throughput. Since SLIM is an imaging modality, increasing the throughput of the measurement is straightforward with tradeoffs between the measurement interval, area covered and cell density. The pedestal resonant sensor typically obtains the growth profile of a few cells per measurement, which could be further enhanced by adopting advanced techniques to place single cells on the pedestal sensors. SMR moves a target cell back and forth to measure the growth rate and currently measures one cell at a time. However, it has the potential to measure multiple cells simultaneously by trapping several cells in a single stream and measuring them one by one.

All three methods can be used to characterize the temporal dynamics of cellular response to biochemical stimuli such as changes in nutrient level, addition of growth factor, and exposure to drugs causing cell cycle arrest or apoptosis However, drastic modification to the media could alter its physical properties such as refractive index, density, and viscosity, which could bias any of the mass measurements unless proper precautions are taken and controls experiments are performed. Lastly, with SLIM and the pedestal resonant sensors, the cells can be easily analyzed with immunostaining or confocal microscopy after the growth rate characterization.

In summary, significant advances have been made in recent years to measure the mass and related physical properties of single cells. Important insights have been gained in the growth trends of mammalian cells, especially in understanding the link between cell cycle progression and mass. Each technique has its own unique potential for further advancement, especially in the simultaneous measurement of additional properties such as protein expression (fluorescence markers), cell stiffness, cell density, and refractive index. For example, it has been established that stiffness of the environment can modulate cell function and differentiation,[37] and that cancer cells are softer than non-cancer cells.[38] These findings indicate that cells are highly sensitive to mechanical cues from its environment. The measurement of cell stiffness over the cell cycle or in context of the mass growth could shed insights on important biological questions, for example, whether the stiffness and growth rate are regulated or coordinated in any manner during the escape of metastatic cells from a primary tumor. Considering the broad impacts of such measurements, it is not surprising that we are already seeing active research efforts toward this direction.[33] Undoubtedly, advancements in single cell optical or mechanical measurement technologies can help to advance the state of understanding of important biological phenomena and also possibly develop new diagnostic or screening assays.

Author contributions

G.P. and R.B. initiated the writing of the article and all authors contributed to the manuscript.

Competing financial interests

GP has financial interests in Phi Optics, Inc., a company that commercializes quantitative phase microscopes. However, Phi Optics did not sponsor this research. The QPI and the resonant pedestal mass sensing approaches have been reported on by the authors in prior publications.

References

1 P. Fantes and P. Nurse, *Exp. Cell Res.*, 1977, **107**, 377–386.
2 P. Nurse, P. Thuriaux and K. Nasmyth, *Mol. Gen. Genet.*, 1976, **146**, 167–178.
3 J. B. Weitzman, *J. Biol. Chem.*, 2003, **2**, 3.
4 I. Conlon and M. Raff, *J. Biol. Chem.*, 2003, **2**, 7.
5 I. J. Conlon, G. A. Dunn, A. W. Mudge and M. C. Raff, *Nat. Cell Biol.*, 2001, **3**, 918–921.
6 I. J. Conlon, L. L. Cheng, G. Dunn, A. Mudge and M. C. Raff, *Mol. Biol. Cell*, 2000, **11**, 345A.

7 S. Cooper, *BMC Cell Biol.*, 2004, 5.
8 S. Cooper, *Theor. Biol. Med. Modell.*, 2006, 3.
9 A. Tzur, R. Kafri, V. S. LeBleu, G. Lahav and M. W. Kirschner, *Science*, 2009, **325**, 167–171.
10 J. F. Collins and M. H. Richmond, *J. Gen. Microbiol.*, 1962, **28**, 15–33.
11 M. Butler, *Appl. Microbiol. Biotechnol.*, 2005, **68**, 283–291.
12 M. Laplante and D. M. Sabatini, *Cell*, 2012, **149**, 274–293.
13 K. Inoki, M. N. Corradetti and K.-L. Guan, *Nat. Genet.*, 2004, **37**, 19–24.
14 J. M. Mitchison, *Theor. Biol. Med. Modell.*, 2005, **2**.
15 H. G. Davies and M. H. Wilkins, *Nature*, 1952, **169**, 541.
16 R. Barer, *Nature*, 1952, **169**, 366–367.
17 G. Popescu, Y. Park, N. Lue, C. Best-Popescu, L. Deflores, R. R. Dasari, M. S. Feld and K. Badizadegan, *Am. J. Physiol.: Cell Physiol.*, 2008, **295**, C538–C544.
18 G. Popescu, *Quantitative phase imaging of cells and tissues*, McGraw-Hill, New York, 2011.
19 M. Mir, Z. Wang, Z. Shen, M. Bednarz, R. Bashir, I. Golding, S. G. Prasanth and G. Popescu, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 13124–13129.
20 S. Sridharan, M. Mir and G. Popescu, *Biomed. Opt. Express*, 2011, **2**, 2815–2820.
21 M. Godin, F. F. Delgado, S. Son, W. H. Grover, A. K. Bryan, A. Tzur, P. Jorgensen, K. Payer, A. D. Grossman, M. W. Kirschner and S. R. Manalis, *Nat. Methods*, 2010, **7**, 387–390.
22 T. P. Burg, M. Godin, S. M. Knudsen, W. Shen, G. Carlson, J. S. Foster, K. Babcock and S. R. Manalis, *Nature*, 2007, **446**, 1066–1069.
23 A. K. Bryan, A. Goranov, A. Amon and S. R. Manalis, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 999–1004.
24 W. H. Grover, A. K. Bryan, M. Diez-Silva, S. Suresh, J. M. Higgins and S. R. Manalis, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 10992–10996.
25 S. Son, A. Tzur, Y. Weng, P. Jorgensen, J. Kim, M. W. Kirschner and S. R. Manalis, *Nat. Methods*, 2012, **9**, 910–912.
26 K. Park, L. J. Millet, N. Kim, H. Li, X. Jin, G. Popescua, N. R. Aluru, K. J. Hsia and R. Bashir, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 20691–20696.
27 K. Park, J. Jang, D. Irimia, J. Sturgis, J. Lee, J. P. Robinson, M. Toner and R. Bashir, *Lab Chip*, 2008, **8**, 1034–1041.
28 M. Terasaki and M. Dailey, in *Handbook of biological confocal microscopy*, Springer, 1995, pp. 327–346.
29 L. Schermelleh, R. Heintzmann and H. Leonhardt, *J. Cell Biol.*, 2010, **190**, 165–175.
30 R. Wang, Z. Wang, L. Millet, M. U. Gillette, A. J. Levine and G. Popescu, *Opt. Express*, 2011, **19**, 20571–20579.
31 R. Wang, Z. Wang, J. Leigh, N. Sobh, L. Millet, M. U. Gillette, A. J. Levine and G. Popescu, *J. Phys.: Condens. Matter*, 2011, **23**, 374107.
32 K. L. Cooper, S. Oh, Y. Sung, R. R. Dasari, M. W. Kirschner and C. J. Tabin, *Nature*, 2013, **495**, 375–378.
33 S. Byun, S. Son, D. Amodei, N. Cermak, J. Shaw, J. H. Kang, V. C. Hecht, M. M. Winslow, T. Jacks, P. Mallick and S. R. Manalis, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 7580–7585.
34 K. Park and B. Rashid, *presented in part at the 15th International Conference on Solid-State Sensors, Actuators, and Microsystems*, Denver, Colorado, 21 June–25 June 2009, 2009.
35 K. Park, N. Kim, D. T. Morisette, N. R. Aluru and R. Bashir, *J. Microelectromech. Syst.*, 2012, **21**, 702–711.
36 B. Bhaduri, D. Wickland, R. Wang, V. Chan, R. Bashir and G. Popescu, *PLoS One*, 2013, **8**, e56930.
37 D. Discher, P. Janmey and Y. L. Wang, *Science*, 2005, **310**, 1139–1143.
38 S. E. Cross, Y. S. Jin, J. Rao and J. K. Gimzewski, *Nat. Nanotechnol.*, 2007, **2**, 780–783.