Opto-mechanical platforms for cell force study

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Microsystems are providing key advances in studying single-cell mechanical behaviours. The mechanical interaction of cells with their extracellular matrix is fundamentally important for cell migration, division, phagocytosis and apoptosis. As the displacement and scales of cellular phenomena is comparable to optical wavelength, optical metrology offers superior resolution and real-time imaging capabilities to measure cell forces and subcellular behaviour as compared to its traditional counterparts. This review Letter discusses the principles, formation and methodological aspects of building opto-mechanical systems in studying cell forces. The authors report current advances of various opto-mechanical systems in studying different aspects of cell mechanics.

1. Introduction: Cells are complex entities in that they not only sense chemical cues, but also interact with their living environment mechanically for cell division, growth, phagocytosis, apoptosis and migration [1–5]. They respond to stimuli whose effects may be beneficial (e.g. in natural or engineered nutritive microenvironments) or harmful (e.g. in aging, stress or injury) to human health. It is therefore a crucial factor in physiological processes such as development and wound healing. Bidirectional chemical and mechanical signals between cells and their extracellular matrix result in the regulation of cellular processes and the remodelling of the extracellular environment [6–8]. Driven by applications on physiology, medicine as well as basic research in cell biology, the advances of micro- and nanotechnology has enabled breakthroughs in understanding and quantifying the sensory and regulatory mechanism between living entities to their immediate living environment, and cell behaviour including transduction, adhesion, motility and proliferation. These biomechanical transductions, expressed in physical terms as mechanical forces, usually ranged from pN to nN, is critical in developing micro/nanoimplantable tissues and scaffolds, and sensors and medical devices that are able to sense and regulate the biophysical property of living cells, diagnostics of abnormal cellular and subcellular behaviours. The detection of such interactions can serve as a unique index to probe trivial changes in single cells prior to the time when physiologic or morphologic changes can be resolved. Therefore sensing physical interactions and performing cell force measurement requires engineering devices that can resemble the microenvironment, and provide a real-time assessment and diagnosis of cell forces in a controllable environment.

Over the past few decades, the development of micro- and nanotechnology has enabled breakthroughs in understanding and quantifying the role of cell traction forces to cell behaviours. These include micropipette aspiration [9, 10], traction force microscopy [11], atomic force microscopy (AFM) [12–15], laser tweezers [16], magnetic twisting cytometry [17, 18], micropore compression, micropost array detectors [19] and many other MEMS-comb drive-based devices [20, 21]. The major disadvantages of probe-based measurements, such as AFM and optical/magnetic tweezers, are the speed and degree to which a mechanical force exerted by a probe transmits across the cell body. Bead-based approaches also lack in itself that the only the bead itself are tracked leaving the surrounding cellular materials unlabelled. They also rely on decoupling and tracking individual sensing units and deriving forces from their physical displacements. These approaches are limited by having discrete sensing units on the substrates; no existing technique is capable of mapping cell force and cell substrate mechanical interactions using a real-time whole field approach and/or sub-cellular components. Optical methods offer significant advantages on mechanical measurements. Remote, non-contact measurement, high spatial resolution and sensitivity and its whole field capacity of visualisation of mechanical signals are the major advantages. Progresses in optical metrology, photomechanics, holography, interferometry and the use of digital image processing have enabled visualisation of sub-cellular behaviours and cell mechanics study in dozens of nanometers and provide real-time and whole field mapping and visualisations. This optical path length information can be readily translated into cell thickness, cell internal structures, having a spatially uniform refractive index. The capability of these methods have been greatly extended by combining them with MEMS polymer and non-polymer periodic substrates that permit live-cell mechanics study and mapping of force-dependent dynamic processes.

In this review Letter, we present new advances in cellular force measurement based on opto-mechanical-based methods and discuss its unique capacities in studying cell mechano-transductions. Optical interference, reflective diffraction gratings and optical moiré methods in combination with cell culture microplatforms are reviewed. The principles, formation and methodological aspects of building opto-mechanical systems in studying different aspects of cellular mechanics are discussed.

According to the types of opto-mechanical-cell interactions, techniques that study cell force can be generally grouped into two categories: active probing technique that applies a mechanical stimulus through optical fields to the cell surface and measure its mechanical response. Passive techniques designed to monitor cell forces utilising optical mapping. Active approach is based on using optical fields/pressure to apply prescribed stimuli onto the cell body and measure its deformability. Optical tweezer-based approaches have been reviewed extensively and implemented by many investigators in studying cell deformability, bulk elastic and viscoelasticity. These optical tweezers (also known as optical trap) utilise light to trap microscopic objects non-invasively. It provides a flexible tool for ultrafine positioning, measurement and control. In practice, forces up to approximately 200 pN may be applied with sub-pN resolution on objects whose characteristic dimensions are similar to the wavelength of light. In cellular force measurement, the laser trap is used to place and hold a fibronectin-coated bead on the cell to monitor cellular force and displacement [22]. The optically induced dielectrophoretic method, developed by Guck et al. [23], has been gaining increasing attention as a non-invasive and high-throughput method to probe cell mechanical property as a compelling supplement to optical trapping. In addition, two-beam optical stretchers consisting of two counter-propagating laser beams have been developed to non-invasive and non-contact stretching of cells and probe their mechanical property. The principle is based on the surface force adding by the two opposed, non-focused laser beams, leading to the stretch of...
biological samples. They have incorporated the optical stretcher with microfluidic interface and utilised the optical deformability to screen different cell phenotype based on their optical deformability [24].

2. Optical interference-based platform: Interferometers provide high resolution and whole field imaging of sample deformations and dynamics by measuring changes in optical path length distributions across the areas to be examined [25, 26]. For two beam with optical path difference $s$ with respect to the other so that the amplitude $U_1 = (I_0)\exp(-jkz)$ and $U_2 = (I_0)\exp[-jk(z-s)]$. The intensity of the sum of the two interference waves can be determined by substituting $I_1 = I_2 = I$ and $k = 2\pi nS/\lambda$ into the interference equation. Therefore the interference intensity where the two waves overlaps is $I = 2I[1 + \cos(2\pi nS/\lambda)]$. Interferometer splits a wave into two waves with equal intensity, delays them by unequal distances, and redirects them using mirrors, recombines them using another beam splitter and detects the intensity of their superposition. Based on the configuration for splitting and recombining the waves, examples of interferometer includes Mach–Zehnder interferometer, Michelson interferometer and Sagnac interferometer [27], and two-beam symmetrical interferometer [27].

Based on the optical configuration, the interferometers have the ability in measuring in-plane, out-of-plane and curvature profiling of the testing samples. We first discuss interference method in determining responses of cell body to the point of applied force in real time. Based on Michelson interferometer, the illumination wavefront can be divided into two separate paths by Michelson interferometer, one transmitted through a reference chamber, one through observation chamber containing cultured cells. Jason et al. [28], demonstrated Michelson interference-based interferometer for studying cell biomechano transduction. In the optical interference technique, the difference in optical path is recorded as a shift in phase between the sample observation and reference beams. A history of optical path length taking together both index of refraction of cell and local thickness can be traced dynamically. In the application described by these authors, NIH 3T3 fibroblast cells were mechanically stimulated by prescribed cyclic magnetic force using a cylindrical rare earth magnet, oriented in perpendicular direction below the chamber (Fig. 1). The magnitude of the applied magnetic force is ranged from 0 to 200 nN at $f = 0.05 \text{ Hz}$ using reflective, magnetic probes attached to the cells. To have lateral shear deformation induced by deforming cells through characterisation of the mechanical properties of the periodic structures. Based on off-axis interference, Zheng and Zhang [31] has reported the combination of off-axis interference with periodic substrates in studying lateral cell forces (Fig. 2a). To have lateral shear deformation on periodic patterns induced by cell contraction, the cells were cultured on deformable grating patterns or periodic grid patterns where each grating line or grid deformable by cells. The symmetrical configuration of the two oblique beams configuration ensures that the out-of-plane variation at each point of the interference region is cancelled out. When the periodic substrates are subjected to cellular forces, periodic lines in $X$ and $Y$ directions are deviated from straightness and the wavefronts of the interfering beams are no longer plane, they are indicated as $w(x, y)$. The complex amplitude of the interfering beams can be described as [27]:

$$U_{1}(x, y) = \exp \left\{ \frac{2\pi}{\lambda} p(x, y) + \frac{2\pi}{\lambda} w(x, y) \right\}$$

(1)

$$U_{2}(x, y) = \exp \left\{ \frac{2\pi}{\lambda} p(x, y) + \frac{2\pi}{\lambda} w(x, y) \right\}$$

(2)

Therefore the according intensity distribution at the substrate can be expressed as:

$$I(x, y) = 2 \left\{ 1 + \cos \left( \frac{4\pi}{d} u(x, y) \right) \right\}$$

(3)

When the two off-axis beam illumination system is angularly misaligned, that is, the incidence angles of the two beams are slightly smaller or larger than the first diffraction order angle, the interference intensity can be derived as

$$I(x, y) = 2 \left\{ 1 + \cos \left( \frac{4\pi}{\lambda} \sin \theta_x + \frac{4\pi}{\lambda} u(x, y) \right) \right\}$$

(4)
where \( \theta \) designates the angle between diffracted orders and the normal of the periodic substrate, \( u \) is the in-plane deformation caused by traction force, \( \lambda \) is the wavelength of the laser source and \( d \) is the spatial periodicity of the periodic substrate. It can be seen that the wavefront deformation caused by out-of-plane displacement does not exist in the intensity distribution. This is because out-of-plane distortion are of the same value and sign in both interfering beams, given the symmetrical arrangement of off-axis interference and, as a result, eliminated at the plane upon diffraction from the periodic substrate. The displacements can be decoded from the fringe patterns without measuring the distortion on the moiré fringes. To compute the contraction force distribution from the moiré maps, Fourier transform was applied to the image. A reverse Fourier transform on the masked data after frequency filtering carries noise-free phase information. Fig. 2b shows the wrapped phase change pattern from the moiré pattern when cell contraction was at its peak on stimulation. The traction force distribution was converted from the phase unwrapping map by utilising the load–displacement relationship characterised from nanoindentation on the polymer periodic substrate (Fig. 2c).

3. Diffraction-based platform: Optical transmission diffraction on cell mechanics study was reported by Gopal et al. [32]. They fabricated nanogratings consisting of flexure folding beams suspended between two parallel cantilevers with known stiffness. The probe displacement was measured through grating transmission spectrum, by tracking the position of the diffraction order. The force sensing element is a transmissive grating that is suspended by supporting beams and can be expanded by application of external load along the principle axis of the grating (Figs. 3a and b). The expansion changes the pitch of the grating, leading to a change in the position of diffraction orders. The probe displacement is measured by tracking the change of diffraction order position of the grating pattern. The force is converted from the displacement through mechanical characterisation of the cell probing system. In their system, the force sensor can be treated as a linear spring–mass–dashpot system that consists of the supporting beams connecting with the suspended structure to the anchors, and the flexures that connect adjacent grating beams. Therefore the dynamic properties can be studied through Eigen function analysis. During the experiment, the force sensor was aligned to the cell and mounted on a piezoeactuator which can be used to prescribe mechanical stimulation to the cells. The amount of force applied to the cell can therefore be determined from the diffraction spots captured using CCD camera. The sensitivity of the displacement sensor was determined to be 8 \( \mu \)m/\( \mu \)N from the stiffness of the beam structure. They deformed the membrane up to \( \sim 5\% \) of its original shape to derive the elastic modulus of the PC12 cell that were cultured on a collagen-coated PDMS substrate and mechanically stimulated. The magnitude of forces was detected from the diffraction spots captured using CCD camera. The authors have characterised that the Young’s modulus of the cells cultured on collagen substrate had an average modulus of 425 \( \pm 30 \) Pa, whereas those treated with NGF had a modulus of 675 \( \pm 25 \) Pa. This indicates that the elasticity of the cell was regulated by treating with Nerve Growth Factors and this technique has high spatial–temporal force sensing resolution in sensing the elastic behaviour changes during cell growth. The close synergy between the measurement of diffraction and cellular scale neurological verification would offer insights on the effect of the external conditions on the mechanical properties of cells during growth and differentiations.

Another application of using diffraction fringe patterns is to introduce a reference periodic substrate with same periodicity of the cell culture grating to produce the well-known moiré phenomena. Moiré patterns are a well-known phenomenon in nature that occurs when repetitive structures (such as screens, grids or gratings) are superposed. This produces a pattern of alternating dark and bright areas that can be observed clearly. Moiré techniques have been adapted by scientists and engineers for a vast number of applications in strain analysis, small angles, displacements or movement metrology because of their extreme sensitivity to the slightest displacements, variations or distortions in the overlaid structures, and
their convenience of automatic displacement contouring [33–37]. Inspired by this phenomenon, Zheng et al. [31, 38, 39] has reported a novel cell contractility mapping transducer utilising moiré patterns as a visual and quantitative tool. Coherent light doubly diffracted from two closely placed microfabricated periodic substrates produces diffraction moiré pattern that magnifies the deformation of the testing periodic substrate angular misaligned with the reference grating, with the contrast varying periodically along the optical axis of the system. With small misaligned angle approximation, the diffracted moiré pattern can be treated as interference between self-image of the first grating and the grating with cultured cells (Figs. 4a and b). The in-plane distortion on periodic substrate caused by cell lateral traction forces is magnified by the moiré fringe patterns with magnification factor \( M \) dependent on the misaligned angle between the two substrates.

By integrating cell culture environment and automated fringe analysis, the moiré pattern generated through microfabricated periodic substrates enables the mapping of lateral cell force distribution through phase changes encoded in carrier fringe patterns. This technique can be applied onto existing cell traction force measurement substrates, providing direct contours of displacement and strain distribution as compared to previous direct calculation of the traction fields. The in-plane deformation on the periodic substrate is encoded in the phase carried by carrier moiré fringe patterns, without measuring the distortion of the pattern as compared to previous techniques such as beads tracking and micropost arrays. The fringe pattern can be analysed by the optical fringe processing technique so that the results are presented in the desired graphical form. This technique was implemented by the authors in mapping the contraction of vascular smooth muscle cells. They studied the moiré pattern evolution during smooth muscle cell spreading and cell contraction in response to agonist and demonstrated that the amount of cell contraction in response to agonist is closely related to level of cell traction forces already established in cells. They examined the cell force evolution through moiré pattern evolution and investigated the contractile forces in response to agonist (Fig. 4c). The force exerted by cell traction can then be conveniently studied through moiré pattern evolution. Moiré pattern operations such as addiction, subtraction and multiplication offered simplicity of retrieving terms such as force changes induced by external factors or strain/stress distributions on the substrate. The magnification effect of optical moiré allows force mapping of multiple cells in the whole field of view as compared to previous techniques of mapping individual cells per frame. Abnormality of contraction forces can be conveniently distinguished from moiré fringe distortions [39].

4. Conclusion: In this Letter, we reviewed current advances in opto-mechanical approaches for studying cell mechanics. We described the formations of various approaches based on optical path length difference, diffraction and moiré phenomena in determining lateral and vertical displacement field. Integrating interferometric metrology, diffraction gratings, moiré measurement with periodi cell culture chips would provide non-invasive, real-time and whole field nature of optical encoding makes monitoring living cell mechanics.

5 References

Figure 4 Moiré setup, system and pattern evolution
a Schematic illustration of double-diffraction moiré setup for cell lateral force measurement
b Integration of the diffraction moiré system with cell culture environment
Cell patterning and moiré pattern evolution:
c (front top row to bottom row) Vascular smooth muscle cell just seeded on the substrate; Cell spread-out and traction forces were generated; Cell relaxed in serum free media and traction forces were reduced


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