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The future of traction force microscopy

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Abstract

Animal cells continuously sense and respond to mechanical force. Quantifying these forces remains a major challenge in bioengineering; yet such measurements are essential for the understanding of cellular function. Traction force microscopy is one of the most successful and broadly-used force probing technologies, chosen for the simplicity of its implementation, flexibility to mimic cellular conditions, and well-established analysis pipe-line. Here, we review the accomplishments, and discuss the applicability and limitations of traction force microscopy. We explain fundamental shortcomings of the method, summarise latest improvements, and outline future pathways towards the impact of the method, especially considering latest developments in state-of-the-art super-resolution fluorescence imaging. In light of the increasing discovery of the importance of mechanobiology in cell physiology, we envisage traction force microscopy to remain a major player for quantifying mechanical forces in living cells.

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Introduction

New perspective of mechanobiology is currently emerging across multiple disciplines in biomedical research. In contrast to conventional beliefs, recent evidence indicates that cells regulate their cell mechanics not only downstream of signalling events triggered by external stimuli or ligand–receptor binding, but that cells employ a diversity of feedback mechanisms enabling them to dynamically adjust their mechanics to meet physiological needs [1,2]. Consequently, this provides a previously unforeseen picture wherein cells actively exert and resist force to tune their material properties and thus facilitate their function, which is particularly important during physical interactions with other cells or with the extracellular environment [3,4]. Quantifying these cellular forces has therefore become an important mission across multiple disciplines at the interface of biophysics, cell-biology, and immunology [5–7].

Measuring these cellular forces is challenging but the methodology of traction force microscopy (TFM) is likely to remain the leading force probing technology. In addition to the complexity of the mechanical feedback mechanisms of cell mechanics, cellular force probing is itself inherently challenging because of the physics of mechanical force measurement. Because cells do not emit mechanical signals that could be detected and analysed in a contactless manner, such quantification demands direct engagement of the force probing technology with the cell. For example, the mechanical stiffness of cells in the form of the elastic modulus is determined by physically indenting their surface by a given force using e.g. atomic force microscopy [6,8]. In TFM experiments, cellular force production is quantified by monitoring surface tractions produced by cells onto an elastic substrate of a given elasticity [9–11]. To add further complexity to this picture, cell mechanical measurements also depend on how they are executed. Especially, cell rheology, time-dependent mechanical properties, vastly differs at different time- and length-scales. On short time-scales (milli-seconds) and large length-scales (micro-meters) cells show poroelastic properties, and at long time-scales (~minutes) they exhibit a power law behaviour in response to application of external forces [12,13]. Hence, parameters such as displacements, cell tractions, and turnover rates must be monitored at a multitude of time- and length-scales in order to comprehensively characterise cell mechanical properties and force production.

Traction force microscopy

TFM is perhaps the most successful and broadly-used force probing technology, because it continues to offer the majority of the above discussed requirements for the quantification of force production in living cells [14,15]. Consequently, TFM is superior to other force quantification technologies due to its simplicity of

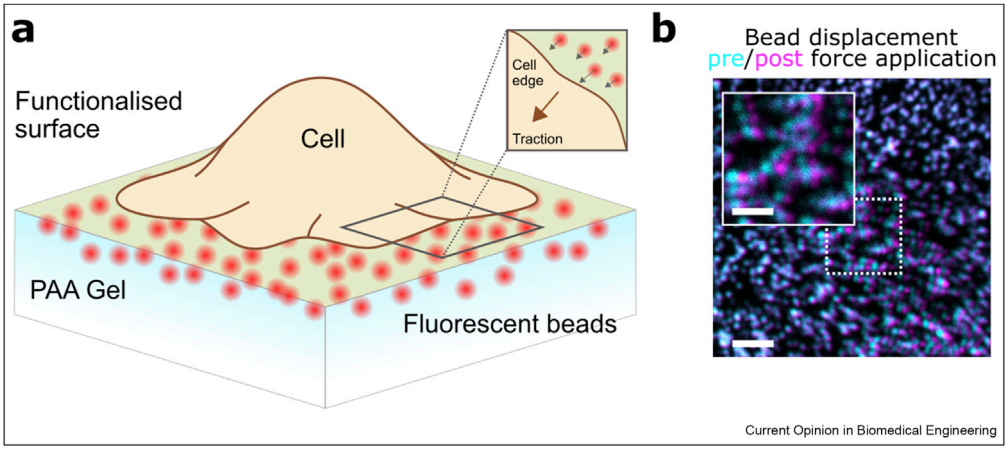
implementation, flexibility to mimic cellular conditions, and well-established analysis pipe-line. In TFM experiments, cells interact with a thin (20–30 μm) elastic hydrogel by adhering to a protein functionalised surface [16,17]. Within the hydrogel, immobilised fluorescent beads serve as fiducial markers, and imaging of the bead positions over time in two or three dimensions (2D/3D) during the application of cellular tractions allows the 2D, 3D elastic displacement of the gel to be quantified. Combining the displacement measurements with knowledge about the mechanical properties of the hydrogel allows the forces applied by the cell to be recovered [14] (see Figures 1 and 2).

Implementation of conventional TFM experiments can be achieved at any confocal or epifluorescence microscope without additional optical components [15]. TFM elastic substrates, such as the often used polyacrylamide (PAA) hydrogels, can be fabricated with elasticities in a range extending from <1 kPa to a few hundreds of kPa, allowing TFM measurements the flexibility to imitate a multitude of different cell surface stiffnesses and tissue environments [14]. In addition, nano-topological features, such as the gel mesh-size can be straightforwardly altered by shifting the balance of monomers and crosslinkers within the PAA gel, without changing the gel stiffness [18]. Alternative materials such as silicon, collagen, and polydimethylsiloxane, known as PDMS, exhibit similar optical and elastic properties, and are considered promising candidates for force probing using TFM-like experiments [19–21]. The refractive index of silicon hydrogels matches the index of the sample coverglass and therefore allows total internal reflection fluorescence (TIRF)-based TFM, which is naturally limited to 2D tractions due to the TIRF imaging [20]. Collagen-based 3D TFM promises the possibility to suspend cells within physiological 3D microenvironments, but its analysis still demands complex algorithms due to its non-linear mechanical properties. Specifically, analysis frameworks necessitate in-depth mathematical knowledge and are practically not available to most biologists or biophysicists [22,23]. In contrast, in TFM, the recovery of mechanical forces from the acquired tractions is in most cases well-established and open-source software solutions are widely available also to non-experienced users. Moreover, the hydrogel's top surface can be covalently functionalised with lipids and proteins mimicking physiological conditions with which the cells interact. Together, these properties allow to quantify the production of cellular tractions by a variety of cells at multiple mechanical conditions.

Limitations of TFM

TFM has been successful in quantifying cellular force production but precludes the characterisation of mechanical properties, which naturally limits the method to only one of the two branches of cell mechanics. TFM does not allow active interrogation of cells through e.g. indentations for the quantifications of mechanical properties such as the deformability, viscosity, or stiffness of cells. Interpreting 2D TFM experiments are also fundamentally limited to examination of force at the ventral membrane of cells. 2D TFM can only provide insights into force production of cell compartments that generate tractions on the apical flat surface of the hydrogel. Efforts to extend TFM to 3D hydrogels have shown promising results but at low throughput and hence limited statistical-relevant output, and further necessitate

Figure 1



Traction force microscopy. a) Schematic outlining a typical traction force experiment. A thin (20–30 μm) PAA gel formed on a glass coverslip is loaded with fluorescent marker beads and its top surface functionalised with proteins that facilitate cell adherence. Traction forces generated by the cell result in displacement of the PAA substrate which can be quantified by imaging the displacement of the fluorescent beads within the gel. b) Representative confocal fluorescent image showing bead positions before (cyan) and after (magenta) the application on cellular traction. Scale bar is 2 μm. Inset shows a zoom-in of the dotted region. Scale bar is 1 μm.