



# The continuous evolution of 2D cell-traction forces quantification technology

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Cells generate traction forces by interacting with the extracellular matrix (ECM) during migration, contraction, invasion, and spreading. Cell-traction forces (CTFs) are extremely small but have enormous biological effects. It has been discovered that CTFs serve a crucial role in regulating proliferation, differentiation, wound healing, morphogenesis, angiogenesis, inflammation, and tumor genesis by working together with biochemical signals to maintain a coherent framework for these processes. For the study of cell biology, it is essential to understand the possible effect of CTFs on the various cellular functions and the amount of traction forces that can be generated by cells in their various states. Currently, CTF quantification approaches are either confined to detecting numerous scattered places on the surface of cells or are severely limited in temporal and spatial resolution, both of which are critical for living cells. Obtaining a highly accurate and dynamic mapping of the force distribution across living cells in real time via a simple mathematical technique remains a significant difficulty. This perspective provides a brief overview of recent landmark advances in the measurement of two-dimensional (2D) CTFs, as well as unique ideas for future improvement.

CTFs produced by the interaction of myosin II with actin filaments can be transferred to the ECM through focal adhesions (Figure 1A). Focal adhesions are<sup>2</sup> transmembrane receptor proteins that primarily include integrins, vinculin, talin, and paxillin and are responsible for establishing physical connections between the ECM and the actin cytoskeleton. CTFs are essential in the regulation of several pathological and physiological processes, including proliferation, differentiation, tumorigenesis, morphogenesis, angiogenesis, inflammation, and wound healing. Therefore, quantifying CTFs is essential for a better understanding of these fundamental biological processes, which can contribute to the development of novel technologies for disease diagnosis and drug screening.

Figure 1B depicts the time frame of the representative CTF quantification methods. In 1980, researchers proposed studying cell motility using an elastic silicone substrate and reflecting CTFs through elastic distortion and wrinkling of the substrate.<sup>3</sup> Thereafter, accompanied by the synergistic advancement of optical materials, micro-nano-fabrication, and computer technology, cell-traction force microscopy<sup>4</sup> was developed, which uses an elastic polyacrylamide gel (PAG) substrate to quantify CTFs. Young's modulus of PAG can be adjusted from 1.2 to 100 kPa by varying the ratio of bis-monomers to acrylamide, which has the benefits of transparency, flexibility, high elasticity, and ease of production. Briefly, after the cells are cultured on a PAG substrate and the microscopy images of the substrate deformation are collected, CTFs can be computed. Furthermore, the markers, which typically include two different colored nanobeads, are often implanted on an elastic substrate to reflect the displacement field, hence improving displacement field resolution. This is mostly because the elastic substrate might deform via the influence of CTF action, causing the markers to migrate. The regularization and filtering methods have been effectively developed to efficiently reduce the error of traction inversion. Nevertheless, cell-traction force microscopy should take a reference image of the substrate topography before calculating CTFs. It is also mathematically complex to compute the traction force field directly from the displacement field.

Thus, high-quality displacement fields are essential, and several research teams have developed organic-based micropillar arrays force sensors by altering the form structure of the elastic substrate to demonstrate this.<sup>5</sup> The aspect proportion of the micropillars could be adjusted based on the mold.

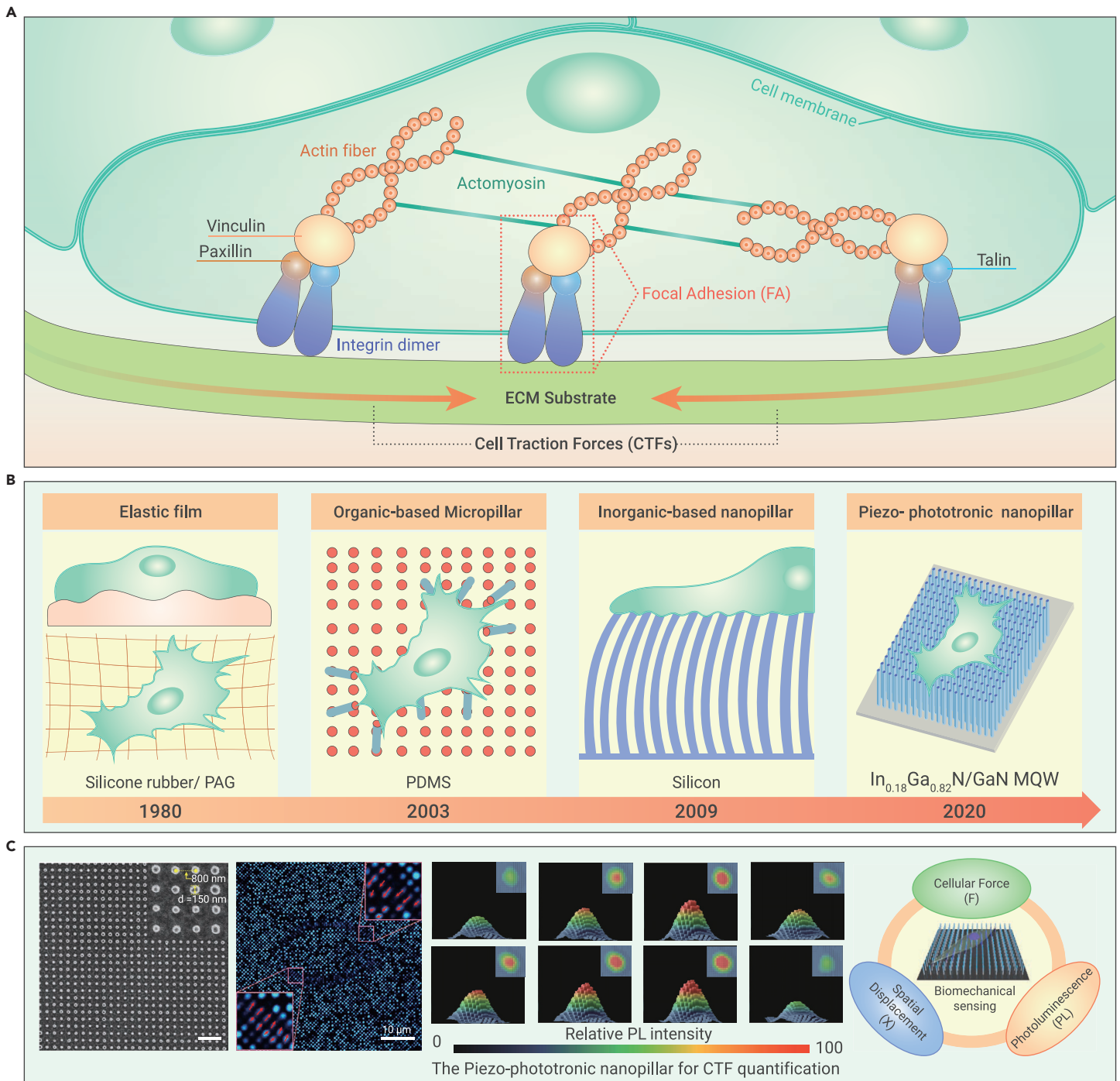
When the micropillar array sensor is functional, each micropillar acts as an independent sensor to quantify the traction force applied by the cells. These micropillar arrays force sensors not only facilitate to measure CTFs in all directions, but they also simplify it significantly to calculate the CTF-induced displacement field. Meanwhile, fluorescent substances or gold nanospheres were modified to be employed as markers on the top of the micropillar arrays to optimize the observation approach. Furthermore, a double-sided micropillar array<sup>6</sup> was also designed for improved precision at low magnifications. However, the spatial resolution of these organic micropillars is rather restricted in the practice by the preparation process. The pillars' diameters are typically measured in microns. By comparison, inorganic pillars developed by the chemical etching of silicon wafers method or photolithography technology can effectively improve the resolution to the sub-micrometer level, but it is impossible to observe the mechanical properties of the living cells in real-time conditions. Typically, the cells are dried and fixed before observing the displacement of the inorganic nanopillars with a scanning electron microscope.

To address the possible drawbacks of the aforementioned measuring approaches, a research team developed a novel InGaN/GaN nanopillar array (1.5  $\mu\text{m}$  in height, 150 nm in diameter) with a high spatial resolution of 31 750 dots per inch (dpi) (800 nm in space between the nanopillars) for quantifying CTFs' distribution in 2020.<sup>1</sup> At the tip of each nanopillar, the multiple quantum well is fabricated, which can be excited by 405 nm light and photoluminescence (PL) at 460 nm. When CTFs are applied to the InGaN/GaN nanopillar array, the inner piezo-potential of the nanopillars will be redistributed, which can further control the PL emission. Furthermore, by employing sapphire as the foundation layer, the device can exhibit improved light transmittance. Therefore, CTFs are directly exhibited by the PL intensities and positions of the nanopillar array based on an optic microscope-laser confocal microscope. This work describes a mechanical-optical coupling approach for real-time CTFs mapping with an ultra-high spatial resolution, high sensitivity, and electric trigger free by using a semiconductor multiple quantum wells matrix through the piezo-phototronic effect (Figure 1C).

CTF quantification technology is constantly being evolved, providing strong support for the research of varied biological impacts, and it can be used as a novel method for disease diagnosis and drug selection. Extending the CTF measurement technique to 3D cell culture can help to further simulate the cell's actual growth environment. Currently, some research groups have successfully developed oil microdroplets<sup>7</sup> and elastic round microgels<sup>8</sup> to obtain 3D CTFs. Both 2D and 3D CTF<sup>9</sup> measurement technologies have distinct application scenarios that can be developed in conjunction. Meanwhile, the DNA-based molecular probes have also been fabricated.<sup>10</sup> The mechanical forces transmitted to specific proteins across cell membranes can be measured more accurately and over longer periods with the use of this technique. Overall, the next stage is to develop CTF measuring technology with high temporal/spatial resolution, accuracy, stability, and real-time capabilities, and its success is dependent on the advancement of novel materials and microfabrication technology.

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**Figure 1. 2D cell-traction force quantification technology (A)** Schematic diagram of CTFs. **(B)** Time frame of the representative CTF quantification methods. **(C)** Real-time and dynamic mapping of CTFs based on piezo-phototronic nanopillar.<sup>1</sup>

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**DECLARATION OF INTERESTS**

The authors declare no competing interests.