31st Annual International Conference of the IEEE EMBS
Minneapolis, Minnesota, USA, September 2-6, 2009

Development of a Versatile Cell Force Transducer using Moiré Mechanism

Xiaoyu Zheng and Xin Zhang, *Member, IEEE*

Abstract— Cells alter their shape and morphology and interact with their surrounding environment. Mechanical forces developed by cells to their surrounding environments are fundamental to many physiological processes, such as cell growth, division, migration, and apoptosis. In this paper, a novel optical moiré based biomechanol force sensor was developed for cell traction force mapping. We utilized coherent laser beams to illuminate periodic polymeric substrates where isolated cells were cultured. We demonstrated one-dimensional and two-dimensional traction force mapping via optical moiré for both cardiac myocytes and vascular smooth muscle cells. The magnification effect of the moiré fringe pattern permits a real time monitoring of the mechanical interaction between isolated cells and their underlying periodic polymeric structures.

I. Introduction

Cell contraction plays important roles in vascular diseases. Contraction forces exerted by cells play essential roles in fundamental physiological processes such as cell growth, division, migration, and apoptosis. Abnormal contraction forces distribution is the earlier causes of vascular diseases including hypertention, atherosclerosis and myocardial infarction.

Many of the previous approaches lack a satisfactory solution for cell force mapping since they require fixation and dehydration and induce undesirable external stimulation to the cells. Therefore such approaches can not render the cell force determination to reveal the contractile state of cells. Recently, micro and nano patterned polymeric substrates have been introduced for measuring cell traction forces [1-6]. These methods introduce a local determination of the traction forces upon discretelized adhesion areas between the cells and their underlying polymeric substrates. These polymeric materials, such as polydimethylsiloxane and polyacrylamide, are biocompatible, and have low elastic modules, allowing for the cell traction force measurements on the order of nN or even smaller. In those techniques, the discrete displacement vectors are derived by tracking and monitoring deflections of each individual micro structure. However, these methods require intensive computation and expertise to track and derive the displacement fields. Moreover, with fixed numerical aperture of the objective lens, the deflection or motion of individual sensing unit is often in the range of sub-micrometers. Tracking such deflection or motion of each individual high-density micro structure is time-consuming, and thus cannot meet the demands for real-time monitoring of cellular and subcellular behaviors.

In this paper, we report the design, characterization and application of a convenient cell contractile forces mapping transducer that can be used to map cell contractility in whole field. This technique utilizes the diffracted moiré pattern from periodic polymer substrate to map the cell contractile state. The cells were cultured on patterned polymer substrate, with a reference layer mounted underneath; the diffracted moiré pattern reveals the variation of in-plane distortion caused by contractility change of the isolated living cell. With this system, we observed moiré patterns revealing vascular smooth muscle cell contraction regulated by contractile agonists. In this paper, we report the development of optical moiré sensing for single cell analysis and its capacity as a mapping transducer.

.

II. Materials and Methods

A. 1-D Optical Moiré mapping

For in-plane cell contraction force mapping, we have

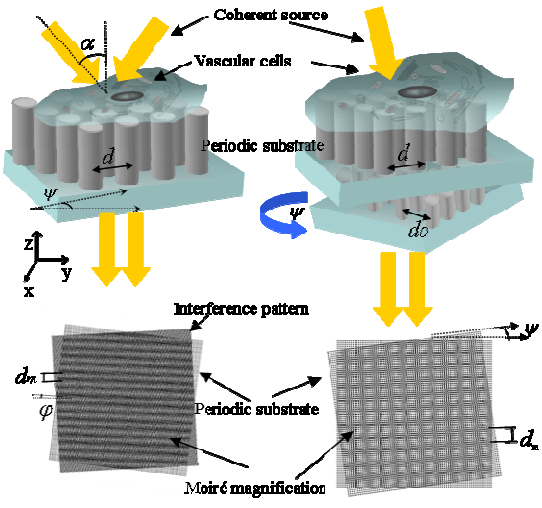

Fig. 1. Schematic illustration of optical moiré scheme for cell traction force mapping. (a) Configuration 1 for 1D force mapping. (b) Configuration 2 for 2D force mapping.

Manuscript received April 7, 2009. This research was supported by NSF CAREER Award (CMMI-0239163) and in part by NSF grants, CMMI-0826191 and CMMI-0609147. Xiaoyu Zheng is with Boston University, Boston, MA 02215 USA (e-mail: raynexzheng@gmail.com)

Xin Zhang is with the Department of Mechanical Engineering, Boston University, Boston, MA 02215 USA. (phone: 617-358-2702; fax: 617-353-5548; e-mail: xinz@bu.edu).

studied two configurations: 1) double-beam interference configuration [Fig.1(a)] and 2) double-periodic layer configuration [Fig.1(b)], for one-dimensional (1D) and two-dimensional (2D) contraction force mappings, respectively. These two configurations share the common feature that in-plane distortion caused by cell contraction forces on periodic substrates is magnified by moiré patterns. The strategy using Configure 1 to map contraction force is rationalized below. As illustrated in Fig.1 (a), two mutually collimated coherent electromagnetic waves are incident symmetrically at the periodic substrate consisting of arrays of vertical PDMS (polydimethylsiloxane) pillars. When the incidence angles of the two beams are detuned to be slightly smaller or larger than the first diffraction order angle, carrier interference fringes are introduced, i.e., given θ_x, the angle between diffracted orders and the normal of the periodic substrate, λ the wavelength of the laser source and d the spatial periodicity of the periodic substrate, the interference intensity can be expressed as

$$I(x,y) = 2[1+\cos(\frac{4\pi}{\lambda}\sin\theta_x x + \frac{4\pi}{d}u)] \quad (1)$$

It can be seen that for the symmetry of the two beams, wavefront deformation caused by the in-plane distortion of the periodic substrate is reserved, whereas the out-of-plane component caused by cell topography changes is of the same value and sign in both interfering beams at their interference location and, as a result, are eliminated by the interference of the two beams. Furthermore, when the orientation of the spatial periodicity of the substrate is not aligned in either X or Y directions, but instead with a denoted angle θ_x, the frequency vector of the periodicity in Fourier space of the diffraction pattern bears on the angle of incident beams and the periodicity of the substrate, namely

$$\frac{1}{d_m}\exp(-j\varphi) = \frac{2\sin\alpha}{\lambda}\exp(-j\psi) - \frac{1}{d}, \quad (2)$$

where d_m and φ are the spatial period and orientation of the diffracted moiré fringes, respectively. α is the angle between the two mutually coherent beams, d is the periodicity of the substrate which superimposes with the two beams. If we tune the angle of the beams to make the spatial period of the interference pattern be identical to that of the periodic substrate, and adjust the orientation of the periodic substrate to make a small angle with respect to the interference pattern, via Rayleigh approximation[7], Eq. (1) can be simplified as $d_m=d/\psi$, where ψ denotes the angle between the periodic substrate and the interference pattern. It can be seen that the spatial period of the substrates has been magnified through the moiré patterns. After characterizing the spring constant of the periodic substrates, the contraction forces can be further mapped based on the deformation of the moiré patterns. **Table** listed the parameters for 1-D optical moiré interferometry, with wider intersection angle between the two beams, the interference pattern can be used to interact with periodic patterns with a periodicity of hundreds of nanometers.

θ	Angle of Intersection(θ)	G Spatial period
1/10,000	2/10,000	3.3mm
1/1000	2/1000	0.31mm
1/100	2/100	0.031mm
1°	2	0.0181mm
5°	10	3600nm
20°	40	925nm
40°	80	493nm
60°	120	365nm
80°	160	321nm

Table Orientation and spatial period of moiré pattern for 1-D optical moiré mapping system

B. 2-D optical moiré mapping

Configure 1 can be extended to Configure 2 by replacing the double beam interference by a two-dimensional periodic substrate. The coherent laser source produced collimated plane waves incident at the selected angle to the normal of the periodic substrate with cultured living cells, with the first-order diffracted beams being visualized through a camera. A second polymer substrate with a low aspect ratio and high rigidity was mounted parallel to the first one with the same periodicity. As illustrated in Figure 1(b), the moiré fringes are the interference fringes of the beams doubly diffracted from the two separate periodic substrates. In analogy to Configure 1, Eq. (1) can now be rewritten by replacing $\sin\theta/\lambda$ by d_0 which is the periodicity of the first substrate. The same magnification factor y can be obtained by detuning relative orientation between the two periodic substrates.

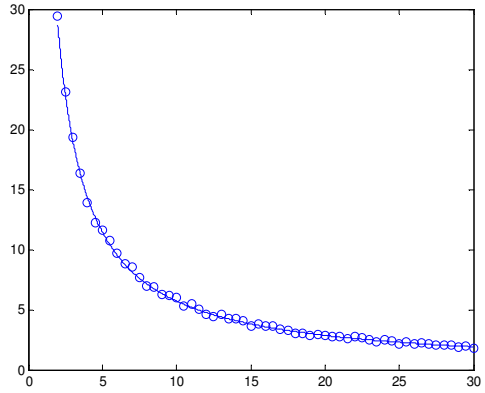

Fig.2. Magnification factor of optical 2D moiré mapping system. Data points represent experimental data; the curve is the theoretical prediction.

Upon registration of two polymeric periodic substrates, the distortion of the moiré pattern is proportional to the distortion of the specimen. We chose the polymeric periodic substrate of the same periodicity but with slightly different aspect ratio. This ensures that the periodicity of the moiré pattern is dependent on only two factors, the periodicity of the substrates and their relative orientation. For 2D moiré pattern, we mounted a second substrate consisting of periodic structures with aspect ratio of 1:3 parallel to the first substrate

and controlled by a precision rotational stage [9]. The moiré pattern is theoretically invariance over the relative translation between two periodic substrates. By increasing the orientation of the second periodic substrate by an interval step of 0.1 degrees, it can be seen that from both the experimental data and the theoretical curves (Fig.2) that the magnification factor decreases with the orientation of the periodic substrate, when the two periodic substrates have the same spatial period.

The displacement resolution in the moiré technique is the tolerance within which a measurement is assumed to be reliable. In moiré fringe technique, what is most important is the relative accuracy of the fringe location, i.e., the relative standard deviation of finding the fringe location in a fringe period. In our study, the absolute standard deviation is highest for a fringe period of six pixels, while the relative standard deviation (dx/dm) to a fringe period is relatively constant for fringe periods of six pixels and higher. This algorithm can determine the fringe location to the accuracy 1/100 of a fringe order. Therefore the displacement resolution is determined to be r=1/100·dm/f, i.e.,1/100·d, where f is the magnification factor of the moiré fringes, dm is the periodicity of the moiré fringes, d is the periodicity of the pillar arrays. For the spatial periodicity of the micro pillar arrays (4μm) employed in our application, the uncertainty of the displacement calculation is about 40nm. However, if the periodicity of the micro pillar arrays is greater than 20μm. The moiré method does not provide higher accuracy over direct imaging method, which gives a resolution around 200nm. Thus, the displacement resolution employed in the moiré technique is mainly determined by the spatial periodicity of the PDMS micro pillar arrays. The smaller the spatial periodicity, the higher resolution it will provide.

III. RESULTS AND DISCUSSION

A. Verification of the magnification factor

To verify the magnification effect of our optical moiré, we performed thermal heating experiments for verification of the

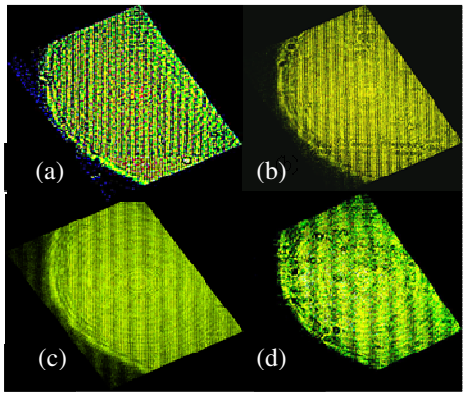

Fig.3. Thermal heating experiment: Moiré pattern generated at a range of temperatures, the wavelength of the laser source is 581 nm.

magnification effect. The measurement was first performed to investigate the small deformation caused by thermal heating. Fig. 3 shows the moiré pattern generated from laser beams as reference gratings and periodic polymeric pillars with periodicity of 8 μm. With temperature changed from 298K to 373K, moiré fringes with different periodicities were obtained as deformation is induced by thermal expansion of the polymeric substrate due to heating. (a), (b), (c), and (d) are moiré patterns generated at temperature of 298K, 323K, 348K, 373K, with moiré fringes expanded at 15 μm, 25 μm, 40 μm, 60 μm, respectively. The angle between periodic structure and interference patterns was chosen at 8 degrees. The expansion of moiré patterns were then used to structure such that thermal strain can be computed. The derive the expansion of the spatial period of polymer periodic corresponding CTE was calculated to be about 3%, which corresponds to literature values [8].

B. 2-D Traction Force Mapping on Vascular Smooth Muscle Cells Spreading

To demonstrate using optical moiré pattern for traction force mapping, in this paper, we demonstrated cell traction force development during vascular smooth muscle cell

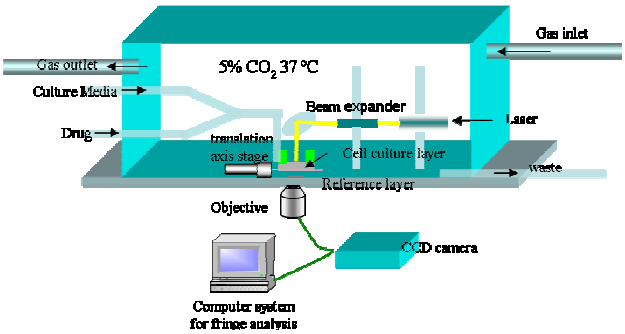

Fig.4. Optical moiré integrated with on chip cell culture system

(VSMC) spreading. Human Aortic smooth muscle cells (Ao184) were grown in Dulbecco's modified Eagle's medium

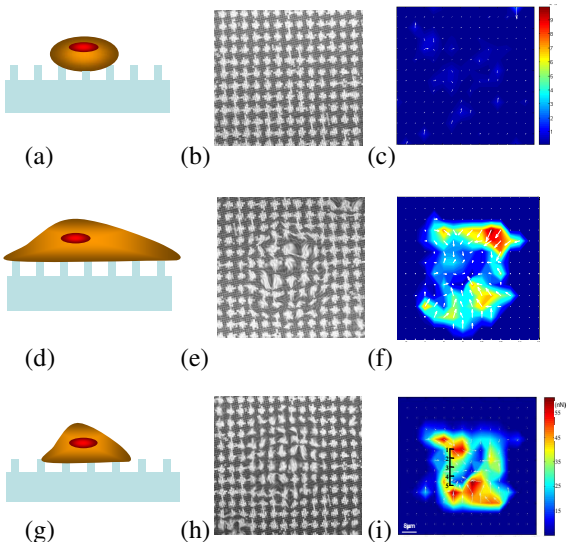

Fig.5. Cell patterning and moiré pattern evolution.

(DMEM) with 10% fetal bovine serum. After trypinized, the

cells were transferred to the PDMS periodic substrates (PPS). In order for the cells to establish focal adhesions on the substrate, the cells were kept in DMEM with serum media for 24 hours before inspection. The PPS for cell culture was then brought and mounted on to the perfusion chamber and placed on the microscope stage. The experimental setup is detailed in Fig 4. The experiments were performed with the bioprocessor heated to 37$\pm$0.2ºC. First, the substrate cultured with VSMCs was focused by an inverted microscope. The second substrate was then raised close to the level of the first one. We observed that from around 300 mm, the contrast of the moiré fringes became clearly visible and the contrast increases as the gap between the two substrates decreases. In this study, 2D moiré pattern was recorded as the VSMCs were incubated in DMEM with 5% calf serum overnight. Fig. 5. shows the moiré pattern evolution as the cells were cultured on PPS and started to establish focal adhesion. Fig.5 (a), (d) and (g) are schematics of cell spreading after being seeded. Fig. 5 (b) is the initial state of moiré pattern immediately after cell being seeded. Fig. 5(e) is the moiré pattern after 10 hours after the cell is cultured. As VSMCs spread out, the moiré pattern changed from regularly distributed to locally distorted and further resembled a natural centrifugal pattern, revealing the concentric profile of the traction forces developed on the substrate. In contrast, after changing the media to DMEM without serum and incubated the VSMCs on the PPS overnight, a relative shrinking of the moiré pattern was observed as shown in Fig.5 (h). As the upper and lower PPS were aligned at about 6°, the periodicity of the moiré pattern is measured to be 10 times larger than that of the periodicity of the PPS (4 mm). And the direction of the moiré pattern distortion is opposite to the direction of the PPS pattern profile. Therefore, by comparing the distorted moiré pattern with the undistorted moiré pattern, the relative distortion contour map of the moiré pattern can be derived and mapped as shown in Fig 5 (c),(f) and (i). The arrows show the relative magnitude and direction of the cell traction forces. Colors show the magnitude of the displacements. The derived force map and moiré patterns indicate consistency between moiré and the force evolution map. The moiré fringes evolution confirmed with that, by culturing cells in serum, the cytoskeleton increases its stress state in medium containing serum until it is fully stressed and spread out, whereas in the absence of serum, the distortion area in the moiré pattern decreases which corresponds to a decrease in the cell-substrate adhesion areas and the decrease in the cytoskeleton stress [8]. The force evolution on the VSMCs was clearly viewed, confirming that the traction force increases due to the increase in cytoskeleton tension dependent on serum, whereas in the absence of serum, cell contractility was low as the spreading of cell decreases due to the decrease of the cytoskeleton tension [9, 10].

CONCLUSION

In summary, we reported an optical moiré approach for real time visualization of cell contraction force mapping. We developed a diffraction optical moiré system for both 1D and 2D mapping of the contraction force based on double-beam interference or double-periodic layer configurations. We demonstrated that by characterizing the moiré patterns to the desired dimensions and orientations, the diffracted moiré patterns are capable of revealing real-time force evolution of living cells cultured on the patterned polymer substrates. The optical moiré system can be readily employed, paving the way for studying cell migration, morphology, motility and many other cell-substrate mechanical interactions on polymer substrates. Since moiré pattern automatically provides contour maps of displacements field, and moiré pattern operation can also be conveniently used to retrieve strain information, which requires neither the tracking/monitoring nor the visibility of each individual pillar, we anticipate that this method will increasingly find more applications in the polymer substrates for a variety of mechanics studies in living cells.

ACKNOWLEDGMENT

The authors would like to thank Dr. Howard Surks at Molecular Cardiology Research Institute, Tufts Medical Center and Dr. Ronglih Liao at Brigham and Women's Hospital, Harvard Medical School for their advice and help on this work.

References

[1]Y. Zhao, C. C. Lim, D. B. Sawyer, R. L. Liao, and X. Zhang, "Cellular force measurements using single-spaced polymeric microstructures: isolating cells from base substrate," *Journal of Micromechanics and Microengineering,* vol. 15, pp. 1649-1656, Sep 2005.

[2]K. A. Beningo and Y. L. Wang, "Flexible substrata for the detection of cellular traction forces," *Trends in Cell Biology,* vol. 12, pp. 79-84, Feb 2002.

[3]N. Q. Balaban, U. S. Schwarz, D. Riveline, P. Goichberg, G. Tzur, I. Sabanay, D. Mahalu, S. Safran, A. Bershadsky, L. Addadi, and B. Geiger, "Force and focal adhesion assembly: a close relationship studied using elastic micropatterned substrates," *Nat Cell Biol,* vol. 3, pp. 466-72, May 2001.

[4]Y. Zhao and X. Zhang, "Cellular mechanics study in cardiac myocytes using PDMS pillars array," *Sensors and Actuators a-Physical,* vol. 125, pp. 398-404, Jan 10 2006.

[5]J. L. Tan, J. Tien, D. M. Pirone, D. S. Gray, K. Bhadriraju, and C. S. Chen, "Cells lying on a bed of microneedles: An approach to isolate mechanical force," *Proceedings of the National Academy of Sciences of the United States of America,* vol. 100, pp. 1484-1489, Feb 18 2003.

[6]O. du Roure, A. Saez, A. Buguin, R. H. Austin, P. Chavrier, P. Siberzan, and B. Ladoux, "Force mapping in epithelial cell migration," *Proceedings of the National Academy of Sciences of the United States of America,* vol. 102, pp. 2390-2395, Feb 15 2005.

[7]O. K. a. I. Glatt, *The Physics of Moire Metrology*. New York: John Wiley and Sons, 1990.

[8]R. M. Ezzell, W. H. Goldmann, N. Wang, N. Parasharama, and D. E. Ingber, "Vinculin promotes cell spreading by mechanically coupling integrins to the cytoskeleton," *Experimental Cell Research,* vol. 231, pp. 14-26, Feb 25 1997.

[9]I. M. Tolic-Norrelykke and N. Wang, "Traction in smooth muscle cells varies with cell spreading," *J Biomech,* vol. 38, pp. 1405-12, Jul 2005.

[10]N. Wang, E. Ostuni, G. M. Whitesides, and D. E. Ingber, "Micropatterning tractional forces in living cells," *Cell Motility and the Cytoskeleton,* vol. 52, pp. 97-106, Jun 2002.