Quantitative imaging of surface deformation on substrata due to cell motility by digital holography

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Abstract: A convenient technique is introduced for quantitatively imaging surface deformation on soft material for cellular adhesion. Quantitative phase microscopy by digital holography is shown to provide efficient and versatile means for quantitatively analyzing cellular motility.

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1 Introduction

Cell-substrate interactions play a crucial role in the migratory behavior of adhesive cells. Some soft polymeric gels such as polyacrylamide (PAA) and silicone rubber are used as flexible substrates to culture various cells to match the mechanical properties of the cells, so that their biological activities can be evaluated. Knowledge of substrate elasticity, for example stiffness, and optical measurement of substrate distortion can be combined to produce estimates of the traction forces of cells and the index change of the substrate. Quantitative phase microscopy (QPM) is a particularly important feature of digital holography microscopy (DHM) [1, 2, 3], because it allows measurement of optical thickness with nanometer-scale accuracy by single-shot, wide-field acquisition, and it yields phase profiles without some of the complications of other phase imaging methods. The phase image is immediately and directly available on calculating the 2-dimensional complex array of the holographic image, and the phase profile conveys quantitative information about the physical thickness and index of refraction of cells and the substrate. We have utilized DH-QPM to measure the Young's modulus of PAA gel [4]. Accurate determination of Young's moduli of these soft gels is essential for obtaining exact responses of the cells to the substrate flexibilities [5]. DHM has also been used to visualize wrinkle formation of biological cells on silicone rubber film [6]. Quantitative measures of surface deformation have been extracted and the cellular traction force has been estimated in a direct and straightforward manner. Non-wrinkling elastics substrate PAA was used as the flexible membrane for cell attachment and the index change of PAA can be analyzed by DHM.

2 Materials and Methods

2.1. DHM setup

The DHM setup used in this work is illustrated in Fig. 1. It consists of a Mach-Zehnder interferometer illuminated with a HeNe laser [1]. The object arm contained a sample stage and a microscope objective lens (MO1) that projected a magnified image of the object onto a CCD camera. The reference arm similarly contained MO2, so that the holographic interference pattern contained fringes due to interference between the diffracted object field and the off-axis reference field. The numerical aperture (NA) of the microscope objectives is 0.25 and the magnification is 10×. The specification of CCD is 1024×768, and the pixel size is 4.65μm. The QPM images were reconstructed from the captured holograms by the angular spectrum method [1]. Aberrations and background distortions of the optical field were minimized by available DHM techniques [1]. LED illumination from above BS1 provided a means of acquiring a bright-field (non-interferometric) microscopy image of the specimen.

2.2. Cells-substratum samples

The samples consisted of fibroblast cells cultured on a thin layer of silicone rubber and PAA gel separately, Fig. 2. The silicone rubber film was prepared as described in Ref. [7]. An approximately 100 μm-thick layer of silicone fluid was spread onto the surface of a Petri dish. Exposure to heat for 1-2s resulted in the formation of ~1 μm thick skin of crosslinked material on top of a lubricant layer of silicone oil on the Petri dish. The PAA gel was made from polyacrylamide prepolymer prepared as described in *Wang Laboratory Protocols.*. The Young's modulus of the gel (Acylamide10%, Bis0.03%) is 6×10^3 N/m^2, measured by DHM

setup.[4] Normal human dermal fibroblasts(NHDF) were rinsed with Hanks' balanced salt solution, released from the substrate by treatment with 0.25% (w/v) trypsin in 2.21 mM ethylenediaminetetraacetic acid, centrifuged and re-suspended in growth medium. Both substrates were sterilized with 70% ethanol and rinsed with sterile phosphate buffered saline prior to cell seeding. Approximately 10^4 cells were seeded onto a Petri dish prepared as described above, culture medium was added, and the Petri dish was covered and incubated at 37 ℃ and 5% CO_2. The culture medium was changed every 48 h.

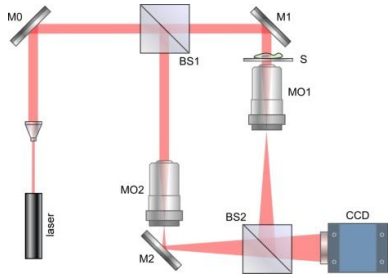

Fig. 1: DHM setup. M's: mirrors; BS's: beam splitters; MO's: microscope objectives (10×); S: sample object.

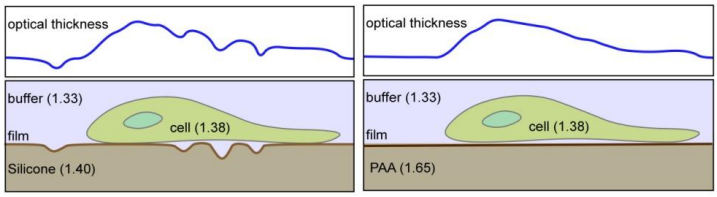

Fig. 2: Schematic of the cell-substrate sample (lower; left: silicone, right: PAA) and the corresponding optical thickness profile (upper).

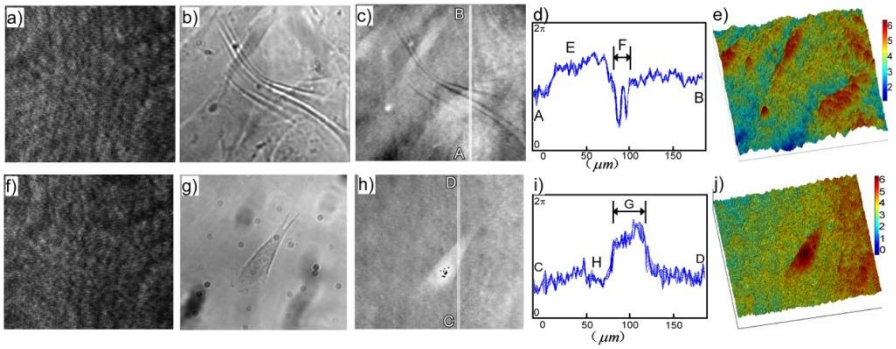

Fig. 3: Examples of cells wrinkling a silicone rubber film (upper panel, a)-e)) and non-wrinkling a PAA gel film (lower panel, f)-j)). The field of view was 190×176 μm^2 with 800×742 pixels. a) & f) Holograms; b) & g) Bright field images; c) & h) Quantitative phase images; d) & i) Cross-sections of phase profiles along highlighted lines AB in c) and CD in h); e) & j) Pseudo-color 3-D rendering of phase images c) & h).

3. Results and Discussions

3.1. Examples of results

Examples of fibroblasts winkling and non-wrinkling a silicone rubber and PAA gel film are presented separately in Fig. 3. Figure 3a) & f) show holograms; Fig. 4b) & g) bright-field images for LED illumination, slightly defocused to make the transparent structures visible; Fig. 3c) & h) quantitative phase images by DH-QPM; Fig. 3d) & i) the optical thickness profiles corresponding to the highlighted vertical line in Fig. 3c) & h); and Fig. 3e) &j) pseudo-color pseudo-3D rendering of the phase images in Fig. 3c) & h). In both cases, the field of view was 190×176 μm^2 with 800×742 pixels. The bright-field image in Fig. 3b) shows several fibroblasts and a few prominent wrinkles, while Fig. 5g) only shows the cell but no wrinkles. In the QPM image in Fig. 3c) & h), the full range of the gray scale values, from black to white, covers the phase variation 0~2π. The cell bodies appear as bright oblong areas because of the higher average refractive index of cytoplasm (~1.38) than buffer (1.33). The wrinkles in Fig. 3c), by contrast,

appear as conspicuous dark lines, indicating that the wrinkles folded into, not out of, the underlying silicone oil layer (1.40). This situation, depicted in Fig. 2, is consistent with the established view [7]. The wrinkles were in general perpendicular to the cell body and the direction of cell motion, as expected for this cell type. The graph in Fig. 3d) & i) are profiles of phase variation along the line AB of Fig. 3c) and CD of Fig. 3h). In fact it plots profiles along ten adjacent vertical lines, to indicate the general noise level. Most of the 'fluctuations' appear to be non-random between adjacent lines, and the noise level is seen to be less than 0.1 radian. The pseudo-3D rendering in Fig. 3e) & j) can provide intuitive visualization of the cells and wrinkling, although one has to use caution interpreting such pictures because the optical thickness represents the combined effect of the physical thickness and the refractive index.

3.2. Phase movie

Time-lapse phase movies of the migration of cells on silicone rubber and PAA gel were recorded every 3 min over a period of 2 hours separately. We focused on individual cells without neighbors in the field of view to minimize the effects of intercellular mechanical interactions through the elastic substrate. In both panels of Fig. 4, an individual cell is seen to spread and crawl on the substratum, changing its shape and orientation. The overall area of the cell increased as it formed protrusions at the leading edge. In the silicone rubber case, the traction force compressed the substratum and stretched it, forming prominent wrinkles in the surrounding area (see arrow). This time-lapse sequence of silicone rubber distortion also indicated that the wrinkles are in general perpendicular to the cell body and the direction of cell motion, as expected. However, the cells in the PAA gel case compressed the substratum without producing any wrinkles, but the compression was seen as darker shadow around the cell body.

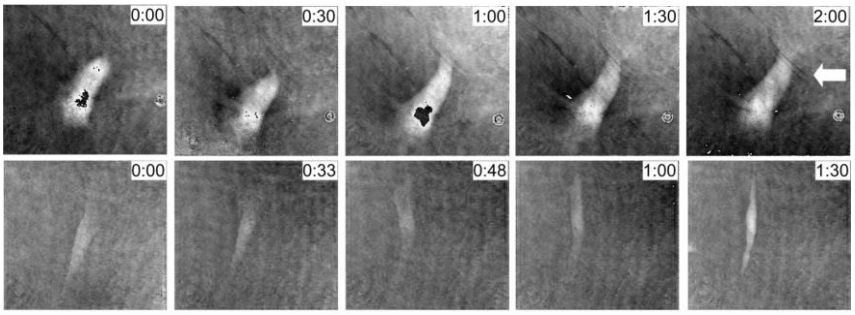

Fig. 4: An excerpt of several frames from phase movie recordings of cells wrinkling a silicone rubber film (upper, Media 1) and non-wrinkling a PAA gel film (lower, Media 2). The field of view was 190×176 μm^2 with 800×742 pixels.

4. Conclusions

The quantitative imaging of surface deformation on silicone rubber and PAA gel due to cells motility has been presented. The basic principles of DH have been applied to quantitative imaging of deformation on substrata due to cell adhesion and motility. The approach is sensitive to cellular movement and it can detect and quantify variations in force within the adhesion area of a cell over time. DH-QPM will be an effective approach for measuring the traction forces of cells and index change of substrata for future study.

Acknowledgments

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