Quantitative imaging and measurement of cell-substrate surface deformation by digital holography

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Abstract: Quantitative phase microscopy by digital holography (DH-QPM) is introduced to study fibroblasts deforming collagen-coated polyacrylamide. Surface deformation has been quantitatively imaged and the traction force of fibroblasts has been measured from phase profiles by DH-QPM.

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The traction forces exerted by fibroblasts cultured on a silicone rubber substrate have been visualized as an elastic distortion and wrinkling by DH-QPM [1]. The traction force has been measured as ~4×10^{-3} dyn/cell based on the degree of wrinkling determined from phase information. Wrinkling is thought to be more elastic than plastic and one may suspect that substrate elasticity will be incomplete and deformation is not entirely linear when forces are applied at multiple locations on the substrate. To address these issues, we applied a non-wrinkling substrate, collagen-coated polyacrylamide (PAA) to make direct measurement of elastic deformations [2] and the Young's modulus of PAA has been measured by DH-[3]. Here, DH-QPM has been used to visualize cell-substrate adhesion and extract quantitative measures of surface deformation. The directly measured substrate stiffness and deformation have been combined to estimate the traction forces.

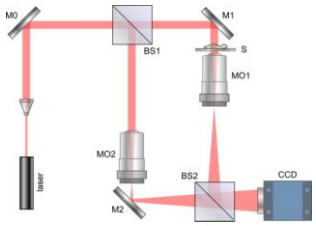

Fig. 1: DHM setup. M's: mirrors; BS's: beam splitters; MO's: microscope objectives; S: sample object

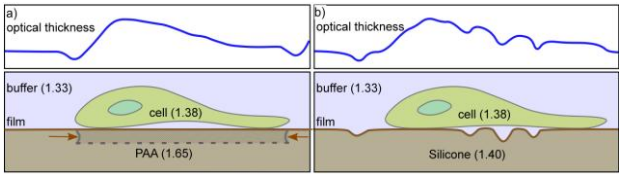

Fig. 2: Schematic of the cell-substrate sample (lower: a) PAA, b) silicone) and the corresponding optical thickness profiles (upper). The cell-silicone sample was taken from Ref [4] as the comparison.

The DHM setup Fig. 1consists of a Mach-Zehnder interferometer illuminated with a HeNe laser, and interference between the diffracted object field and the off-axis reference field results in holographic patterns [1]. The QPM images were reconstructed from the captured holograms by the angular spectrum method. Aberrations and background distortions of the optical field were minimized by available DHM techniques. The samples consisted of fibroblast cells cultured on a thin layer of collagen-coated PAA. The film was made from polyacrylamide prepolymer prepared as described in Ref. [4]. The flexibility of the substrate was manipulated by adjusting the concentrations of acylamide and bis-acylamide. Approximately 10^4 Normal human dermal fibroblasts (NHDF) were seeded onto the coverglass in a Petri dish prepared as described above [1]. The scheme of cells on PAA substrate is shown in Fig. 2a), and an example of cells wrinkling the silicone rubber Fig. 2b) was taken as comparison.

Examples of fibroblasts deforming the PAA gel film are presented in Fig. 3a)-d) and Fig. 3e)-h). The Young's modulus of the PAA substrate are 28kPa and 14kPa respectively, and the thickness are both 78μm. For comparison, an example of fibroblasts wrinkling a silicone rubber film is also presented, Fig. 3i)-l) [1]. Figure 3a), e) & i) show bright-field images for LED illumination. Fig. 3a) & e) shows a single cell crawling on the flat PAA film surface without any wrinkles, while several cells and also a few prominent wrinkles are shown in Fig. 3i). Fig. 3b), f) & j) present quantitative phase images by DH-QPM. The deformation area appears as dark shadow around the cell body because the substrate surface was deformed by certain tangential and vertical displacement and distortion due to the traction forces exerted by cells. Fig. 3c), g) & k) are the optical thickness profiles corresponding to the highlighted lines AB, CD and EF in Fig. 3b), f) & j). G & H are the deformation areas on PAA across the cell body and I is the winkling area of cells on silicone rubber film. Fig. 3d), h) & l) are pseudo-color pseudo-3D rendering of the phase images in Fig. 3b), f) & j), providing intuitive visualization of the cell and substrate.

We measured two sets of the traction forces of cells cultured on the PAA substrate according to the phase variation in the phase profile of the deformation area and the surrounding noise level. The results of cells deformed PAA of various E and thickness are shown in Table 1. The traction forces cells exerted on

PAA substrate are shown to be independent of the thickness but increase with the Young's modulus of the substrate. The traction forces for NHDFs are comparable to literatures since the forces may vary due to the cell type, the physiological state of the cells, substrate and buffer preparation, *etc*.

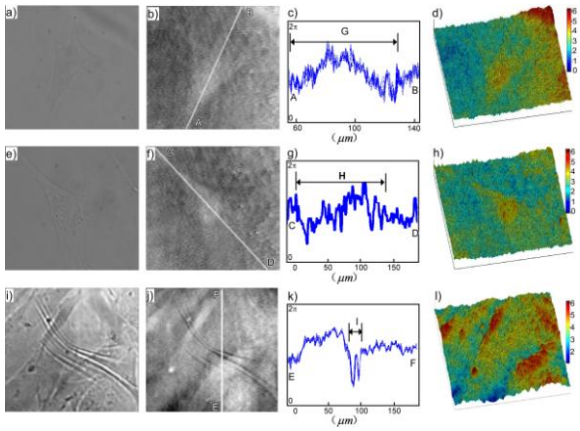

Fig. 3: a)-d) cells deforming a PAA film (Young's modulus of PAA substrate is 28kPa; thickness is 78μm); e)-h) cells deforming a PAA film (Young's modulus of PAA substrate is 14kPa; thickness is 78μm); i)-l) cells wrinkling a silicone rubber film. a), e) & i) Bright field images; b), f) & j) Quantitative phase images; c), g) & k) Cross-sections of phase profiles along highlighted lines AB in b), CD in f) and EF in j); d), h) & l) Pseudo-color 3-D rendering of phase images b), f) & j). The field of view was 190×176 μm^2 with 800×742 pixels.

Table 1 Traction forces on substrate of various Young's modulus (E) and thicknesses.

	Thickness= 40 μm	Thickness= 78 μm	Thickness= 200 μm
E=14kPa	$\Delta\phi = 1.27 \pm 0.64 rad$ $\frac{F}{A_0} = 12.57 \pm 6.18 kdyn/cm^2$	$\Delta\phi = 1.25 \pm 0.63 rad$ $\frac{F}{A_0} = 12.37 \pm 6.18 kdyn/cm^2$	$\Delta\phi = 1.29 \pm 0.50 rad$ $\frac{F}{A_0} = 12.75 \pm 5.19 kdyn/cm^2$
E=28kPa	$\Delta\phi = 1.00 \pm 0.36 rad$ $\frac{F}{A_0} = 20.19 \pm 7.56 kdyn/cm^2$	$\Delta\phi = 0.99 \pm 0.33 rad$ $\frac{F}{A_0} = 20.04 \pm 7.10 kdyn/cm^2$	$\Delta\phi = 1.04 \pm 0.46 rad$ $\frac{F}{A_0} = 20.93 \pm 9.52 kdyn/cm^2$

DH-QPM has been applied to quantitative imaging of fibroblasts deforming a non-wrinkling substrate collagen-coated PAA. The traction force has been measured based on the degree of deformation determined from phase information and shown to be independent of the thickness but increase with the Young's modulus of the substrate. DH-QPM is able to provide direct access to the quantitative measures of the substrate elasticity and sensitive to cellular forces compared to other methods of force estimation, so that it can detect and quantify variations in force within the adhesion area of a cell over time.

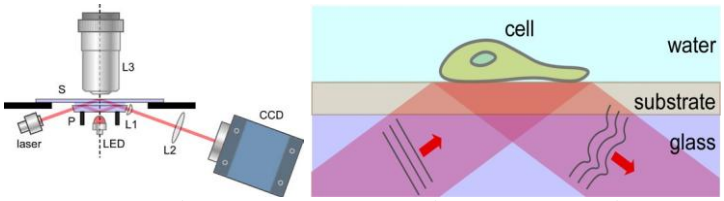

Fig. 4 THM interferometer setup and a detail of the THM prism configuration

Some issues and improvements of the technique are worth mentioning. The overlap of the cell body and intra- and extra-cellular particulate matter in the middle of the deformation would invalidate the phase difference calculation. An improved method total internal reflection digital holography microscopy (THM) is in progress for the better study of cell-substrate interaction, Fig. 4. The substrate of a relatively high index behaves optically as an extension of the glass slide and the prism underneath it. The phase modulation of TIR-reflected light carries the information on the thickness variation of the film due to wrinkling/deformation. The THM image reveals the wrinkle/deformation profiles only, without interference or noise of the cell bodies or spurious debris and inhomogeneities of the buffer solution. THM allow much higher sensitivity measurement and analysis of the surface deformation and lead to observation of subtle effects of cellular motility that may not have been visible before.

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