Measurement of Young's Modulus of Polyacrylamide Gel by Digital Holography

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Abstract: A convenient technique is introduced for measuring the Young's modulus of soft material (polyacrylamide gel) for cellular adhesion with the principles of digital holography.
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1 Introduction

Digital holography (DH) is an emerging technology of new paradigm in general imaging applications and offers a number of significant advantages such as the ability to acquire holograms rapidly, and availability of complete amplitude and phase information of the optical field. The quantitative phase microscopy by digital holography (DH-QPM) has been applied in biomedical field [1]. Interaction between an elastic substrate and adherent cells plays an important role in regulating cellular functions and behaviors in concentration, migration, and invasion. Some soft polymeric gels, such as polyacrylamide(PAA), 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. It has been reported that elastic stiffness of the substrate gels exerts marked influences on mechanical behaviors and biochemical expressions of the adherent cells. Therefore, accurate determination of Young's moduli of these soft gels becomes essential for obtaining exact responses of the cells to the substrate flexibilities [2]. Previous researchers have used methods such as atomic force microscopy (AFM) [3] or manipulation of spherical beads[4] to probe and simulate the stress-strain behavior of the substrate, but these measurements are highly localized and are rather cumbersome. Digital holography is a very effective process for achieving high-precision quantitative phase microscopy. The phase image is immediately and directly available as soon as the 2-D complex array of the holographic image is calculated. The optical phase of the light transmitted through transparent objects can convey quantitative information about the object, such as its physical thickness and index of refraction, which in turn are functions of physical density or chemical concentration properties. Most importantly, the phase image is a quantitative representation of the object profile with nanometer, and even subnanometer precision. Therefore, DH can provide a novel method to measure the Young's modulus of PAA gel.

2 Principles

In the experiment, the PAA gel was made from polyacrylamide prepolymer prepared as described in *Wang Laboratory Protocols, Cover Slip Glass Activation & Polyacrylamide Preparation.* The theoretical value of Young's modulus of the gel made from *Wang Laboratory Protocols* (Acylamide5%, Bis0.1%) is 28×10^3 N/m^2. The gel and disk sample is illustrated in Fig.1.

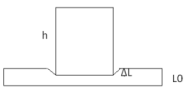


Fig.1.Gel and disk Sample. The thickness of the gel on the cover slip glass (L_0) is 1mm. A solid brass disk with a radius (r) of 2mm, thickness (h) of 0.5mm is placed on the top of the gel surface. The deformation of the gel is shown as ΔL.

The definition of Young's Modulus is in equation (1)

$$E = \frac{stress}{strain} = \frac{\sigma}{\varepsilon} = \frac{F/A_0}{\Delta L/L_0} = \frac{FL_0}{A_0\Delta L} \qquad (1)$$

Where, F is the force applied to the gel, $F = \rho g V$, ρ is the density of the brass disk (=9.7×10^3 kg/m^3), V is the volume ($= \pi r^2 h$). A_0 is the cross-sectional area through which the force is applied ($= \pi r^2$). L_0 is the original length of the object (1mm), ΔL is the deformation change. Then eq.(1) can be simplified as

$$E = \frac{\rho g h L_0}{\Delta L} \quad (2)$$

The deformation of the gel ΔL in eq.(2) is measured by a DH experiment.

The setup of the DH system is shown in Fig.2. It consists of an illumination source, an interferometer, a CCD camera, and a computer with labview programs. The light green beams are the input from the laser, the light blue is the reference beam path, and the red is the object beam path. The object is illuminated with a plane wave, and the reference arrives at the CCD plane with the same wavefront curvature as the object wave, except for an offset in the angle of incidence for off-axis holography. A CCD camera is used to capture and digitize a holographic interference pattern. The captured hologram pattern is digitized by the camera, and input to the computer as a 2-D array of integers with 8-bit grayscale resolution. The main task of the computer is to carry out the numerical diffraction to compute the holographic image as an array of 2-D complex numbers. In addition, the labview program handles a number of other tasks, such as pre- and postprocessing of the images, rendering and storage of images, as well as timing and other necessary controls of the apparatus.

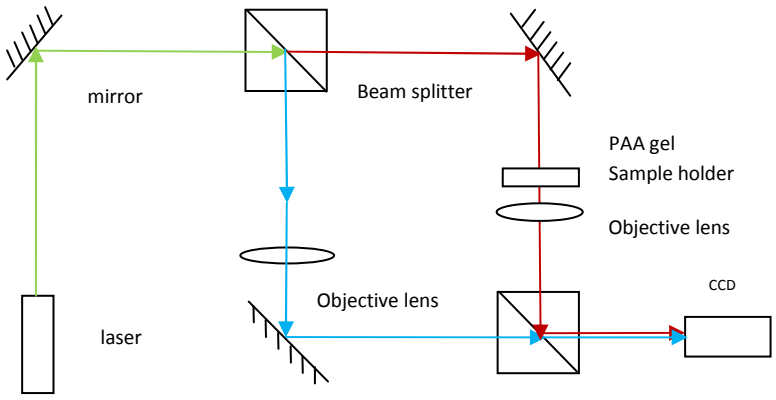
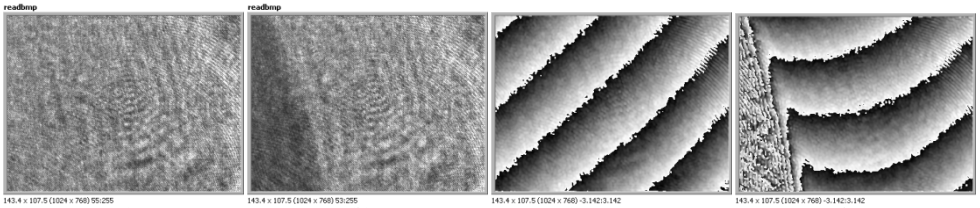


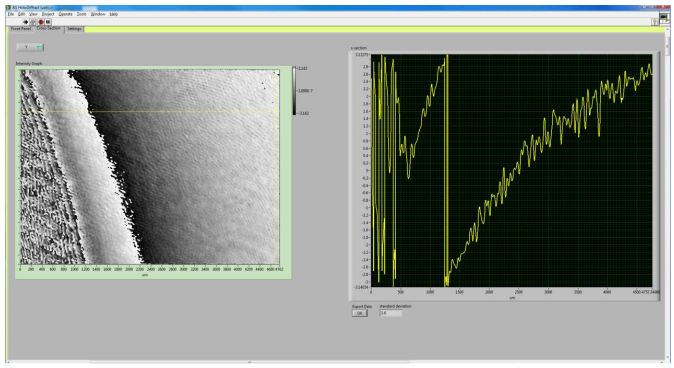
Fig.2. DH system

3 Experimental result and discussion

Fig.3 (a) and Fig.3(c) are the hologram and phase image of the PAA gel sample without a load. Fig.3 (b) and Fig.3 (d) are the hologram and phase image of the PAA gel sample with the brass disk loaded, which partly occludes the left side of the image. Fig.3 (e) is the phase difference between (c) and (d), representing the net deformation of the gel. Fig.3 (f) is a cross-section across the yellow line in (e). The deformation ΔL apparently has thicknesses of several microns, and therefore the phase profile varies by several cycles of 2π radians. A public-domain phase unwrapping algorithm is used to remove the 2π discontinuities. After unwrapping the phase images, we obtained the phase difference of the gel deformation is 1.7 λ, where λ is the wavelength of the laser (=0.633 μm).



(a) (b) (c) (d)



(e) (f)

Fig.3.DHM of gel deformation. (a) hologram of the PAA gel sample; (b) hologram of the PAA gel sample with the brass disk on the left surface; (c) phase image of the PAA gel sample; (d) phase image of the PAA gel sample with the brass disk on the left surface. (e)the phase difference between (c) and (d); (f) the phase scale image.

As shown in Fig.4, the optical path difference is $\left|n_g(d-\Delta L)+n_0\Delta L-n_g d\right|=(n_g-n_0)\Delta L=1.7\lambda$, so that $\Delta L=\dfrac{1.7\lambda}{n_g-n_0}$.

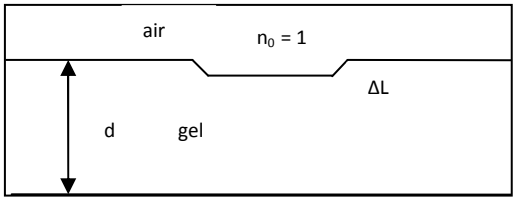


Fig.4. Sketch of gel deformation. n_0 is the refractive index of the air (=1), n_g is the refractive index of the gel, d is the thickness of the gel. ΔL is the deformation of the gel.

The refractive index of gel (n_g) is measured with the Reichert Abbe Mark II PLUS ABBE Refractometer to be $n_g=1.65$, so that the deformation is $\Delta L=\dfrac{1.7\lambda}{n_g-n_0}\approx 1.65\mu m$. Then the Young's modulus of the PAA gel is calculated as $E=\dfrac{\rho g h L_0}{\Delta L}\approx 28.8kN/m^2$. Compared with the expected value $28kN/m^2$ (*Wang Laboratory Protocols* (Acylamide5%, Bis0.1%)), the percent difference is approximately 3%.

4. Conclusions

The basic principles of the digital holography to measure the polyacrylamide gel deformation are demonstrated. Digital holography is a very effective process for achieving high-precision quantitative phase microscopy compared to other methods of measuring deformation of soft materials. The phase image is a quantitative representation of the object profile with nanometer, and even subnanometer precision. Accurate determination of Young's moduli of these soft gels can be used further for exploring the responses of cells (Amoeba) to their substrates (PAA gel).

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