Microscopic Observation of Strain-Induced Birefringence on Macroscopic Deformation Process of Biological Tissue*

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Abstract
Macroscopic deformation of biological tissue shows the various behaviors of nonlinear, aeolotropic and so on. The mechanics of the deformation generally depend on the character of the microscopic cells which have complicated structures like substratum, membrane and wall. In the cells, the strain caused by macroscopic deformation is unevenly distributed because of its structures. However, the structures cause difficulty in the observation of the strain distribution and the study on the deformation mechanics of the tissue. In this study, a birefringence microscope is applied to observe the uneven distribution of the strain. The technique of the microscope with CCD has abilities to evaluate the phase difference and the azimuthal direction, and to observe the value distribution. The uniaxial and biaxial tensile tests and the some considerations are carried out to investigate the deformation mechanics of the tissue of a plant by using the microscope system.

Key words: Biomechanics, Mesoscopic, Optical Measurement, Experimental Mechanics, Stress-Strain Measurement, Biological Soft Tissue, Biaxial Tensile Test, Birefringence Microscopy

1. Introduction

A cell is the basic unit of a biotissue structure and is composed of cytoplasm, a cell membrane, and a cell wall. Considering the deformation of a cell aggregate, we suppose that a tissue is an arrangement of cells that have the same shape and function. The sum of the deformation of individual cells causes a macroscopic deformation of the cell aggregate. However, the phenomenon at the cell level plays an important role in the mechanism of the macroscopic deformation of the cell aggregate. An optical microscope is widely used to observe the deformation at the cell level. However, with respect to observation of transparent objects, a conventional optical microscope is able to visualize only the refractive index distribution and not the changes inside a cell that caused the deformation. Further, a polarization microscope has been used to visualize the molecular orientation and internal stress of a cell. However, the anisotropy of the cell cannot be quantified by visual information. On the other hand, the birefringence microscope used in this study is able to quantify this anisotropy by calculating the birefringence retardation and azimuthal angle. The birefringence microscope employs a CCD camera as a detector, and the analysis values are recorded in each pixel. The purpose of this study is to elucidate the effect of the deformation behavior of a single cell on the macroscopic deformation of a cell aggregate by using the distribution of birefringence retardation.

2. Birefringence Measurement

2.1. Strain birefringence

Birefringence is the optical anisotropy induced by the molecular orientation and the dif-
Strain-induced birefringence in density of a material. In particular, strain birefringence is caused by applying physical or external stress to a material. The refractive index in the direction of stress is larger than that in the direction perpendicular to the stress. Photoelastic measurement, which is the most common method used to measure stress distribution experimentally, utilizes the concept of strain birefringence. On the other hand, birefringence measurement employs a phase shifting method and can quantize birefringence smaller than one fringe; photoelastic measurement cannot measure such amounts of birefringence.

The birefringence-measurement method that employs a phase shifting method is used to detect light intensity whenever the polarization state of the irradiation light changes, and the light intensities are analyzed to determine the birefringence retardation and the azimuthal angle of the sample. This birefringence measurement method has been used to evaluate the quality of transparent injection molding products in the industry\(^2\). Moreover, the local-sampling phase shifting technique was proposed to detect smaller birefringence retardation, and it is especially used to measure the birefringence of the optical elements made of glass\(^3\). The method is possible that light intensity change caused slight phase shift by the birefringence of sample is amplified at the detector virtually. Consequently, a high-accuracy birefringence measurement is realized within a specified dynamic range of a CCD camera. The distribution of the background light and that of the phases by the objective lens and mirror are corrected by phase subtraction. As a result of this correction, the birefringence retardation can be quantified for the entire measurement area\(^1\).

On the other hand, the three-dimensional refractive index of a point has attracted considerable attention in the optical film and injection molding products industries. The measurement of this index is being inspected for polymer products\(^4\)-\(^6\).

2.2. Measurement Method of Birefringence Distribution

2.2.1. Optical Setup

The optical setup for birefringence measurement is illustrated in Fig. 2. This system is equipped with a light source, two polarizers, a quarter-wave plate, a Babinet-Soleil compensator (BSC), and a detector. The polarization state of the light incident to a BSC is circular, and this polarized light is transmitted through a polarizer and a quarter-wave plate. After the polarized light passes through the quarter-wave plate, its polarization state is changed arbitrarily by the BSC. Then, the transmitted light is passed through an analyzer, and its intensity is detected. The detected intensity is analyzed to obtain the retardation and the azimuthal angle of the sample in the following manner. The polarization state of the incident light is expressed by the Stokes parameter S. This parameter is transformed by the Mueller matrix of the optical element, and the Stokes parameter of the transmitted light \(S'\) is obtained. The first component of a Stokes parameter signifies a light intensity. The intensity detected is given by

\[
I_i = \frac{1}{2} I \left[1 - \sin \left(\tan^{-1} (\Delta \cos (2\theta - 2\phi))\right)\right],
\]

where \(\Delta\) represents the birefringence retardation of the sample, and \(\phi\) is its azimuthal
angle. \( \delta \) refers to the retardation given by the BSC, and \( \theta \) is its azimuthal angle. The intensity detected is changed by the retardation and the azimuthal angle of the BSC.

### 2.2.2. Local-sampling phase shifting technique

The part of the equation that includes the retardation and the azimuthal angle of the sample is replaced by phase \( \Phi \theta \)

\[
\Phi \theta = \tan^{-1} (\Delta \cos (2\theta - 2\phi)),
\]

(2)

Substitution of Eq. (2) in Eq. (1) leads to the following equation.

\[
I_i = \alpha (1 + \beta \sin (\Phi \theta + \delta_i))
\]

\[
= a_0 + a_1 \cos \delta_i + a_2 \sin \delta_i,
\]

\[
a_0 = \alpha, \quad a_1 = \alpha \beta \sin \Phi \theta, \quad a_2 = -\alpha \beta \cos \Phi \theta
\]

(3)

If birefringence retardation of sample is small, the difference of the detected light intensity between sample and not sample is small at conventional phase shifting method shown as Fig.3(a). Consequently, a little noise influence on detected light intensity and induce error in the phase detection. In the process of phase detection by using the local-sampling phase shifting technique, the retardation given by the BSC is changed in the range of from \((270^\circ - \alpha)\) to \((270^\circ + \alpha)\). Moreover, the light intensity or sensitivity of the detectors is regulated to make good use of the detection dynamic range. Figure 3(b) shows the light intensity detected by the local-sampling phase shifting technique. Compared to the conventional method, this method detects a high intensity and is capable of a high-resolution detection of the phase.

We obtain the values of \(a_0, a_1,\) and \(a_2\) in Eq. (1) in order to minimize the difference between the detected light intensity and the theoretical light intensity by using the least square method. Applying the matrix notation, Eq. (4) is expressed in the following manner.

\[
\begin{bmatrix}
N & \Sigma_i \cos \delta_i & \Sigma_i \sin \delta_i \\
\Sigma_i \cos \delta_i & \Sigma_i \cos^2 \delta_i & \Sigma_i \sin \delta_i \cos \delta_i \\
\Sigma_i \sin \delta_i & \Sigma_i \sin \delta_i \cos \delta_i & \Sigma_i \sin^2 \delta_i
\end{bmatrix}
\begin{bmatrix}
a_0 \\
a_1 \\
a_2
\end{bmatrix}
=
\begin{bmatrix}
\Sigma_i I'_i \\
\Sigma_i I'_i \cos \delta_i \\
\Sigma_i I'_i \sin \delta_i
\end{bmatrix},
\]

(4)

The phase \( \Phi \theta \) is analyzed by Eqs. (3) and (4). On the condition that the retardation given by the BSC is changed symmetrically with respect to \( 270^\circ \), the phase is expressed in Eq. (5). \( N \) represents the number of times the sampling was carried out.
\[ \Phi_\theta = \tan^{-1} \left( \frac{a_1}{a_2} \right) = \tan^{-1} \left( \frac{N \Sigma_i \sin^2 \delta_i - (\Sigma_i \sin \delta_i)^2 \Sigma_i I'_i \cos \delta_i}{N \Sigma_i' \sin^2 \delta_i - \Sigma_i I'_i \sin \delta_i \Sigma_i \cos^2 \delta_i} \right), \] (5)

The phase \( \Phi_\theta \) is determined from the light intensity by using the local-sampling phase shifting technique. This phase detection is performed when the azimuthal angle of BSC(\( \theta \)) is 0, \( \pi/4 \), \( \pi/2 \), 3\( \pi/4 \). The birefringence retardation \( \Delta \) and the azimuthal angle \( \phi \) of a sample can be calculated by using the equations,

\[ \Delta = \sqrt{(\Phi_{\pi/2} - \Phi_0)^2 + (\Phi_{3\pi/4} - \Phi_{\pi/4})^2}, \] (6)

\[ \phi = \frac{1}{2} \tan^{-1} \frac{\Phi_{3\pi/4} - \Phi_{\pi/4}}{\Phi_0 - \Phi_{\pi/2}}, \] (7)

3. Observation of Deformation Process of Biological Tissue

3.1. Birefringence Microscope

Figure (4) shows the birefringence microscope. The system is equipped with a 635nm Super Luminescent Diode (SLD), elements for birefringence measurement, and a sample stage for installing the biaxial tensile test machine. The microscope can be attached to an objective lens with 5-50 magnification.

![Birefringence microscope](Fig. 4)

3.2. Biaxial Tensile Test Machine

It is important that the experimental environment and the tensile test machine be suited to observe a biological tissue. The observation using a biaxial tensile test in an environment similar to a living body has been reported(7). In this study, a biaxial tensile test machine that can be installed in a birefringence microscope and be used to perform general tensile tests has been developed. A picture of the biaxial tensile test machine is shown Fig. 5(a), and its schematic diagram is shown Fig. 5(b).

The size of the proposed biaxial tensile test machine is less than 100mm × 120mm since the machine has to be installed in a microscope. The sample to be observed is attached to the center of the machine at a number of points in order to avoid restricting the deformation of the sample by using glue and an aluminum foil that is slit into strips. The foil is fixed at four points that are far from the center of the sample so that it does not obstruct the observation.
Moreover, the foil is fixed to two pairs of linear stages by screws. In order to maintain the center, the opposite pair of stages uses a pulley mechanism that is made of the SUS301 foil. Each stage is controlled by an independent small servo motor (Futaba S3103). The tensile test machine can arbitrarily load uniaxial or biaxial tensile stress. The tensile test machine allows a movable displacement of 6.3 mm and a minimum displacement of 0.005 mm.

3.3. Experimental condition

An onion cell was used as a sample in this study since the acquisition of this thin layer tissue is easy. A slice measuring 10 mm × 10 mm was cut from the thin layer tissue. The onion cell is elliptical in shape and has major axis and minor axis. The major axis was defined as the x-axis. The displacement directions to specimen are major axis (a), minor axis (b) and both axes(c) as shown in Fig.6. In this case, the distance between the tips of the aluminum foil on the diagonal line is 5 mm. The measurement area is 1 mm × 1 mm of the center of the sample and is indicated by the square in the figure. An objective lens with a magnification of 10 is used, and the spatial resolution is 1.7 μm.

3.4. Results and Discussion

3.4.1. Uniaxial tensile stress for major axis

The birefringence retardation distribution in the case of loading stress to the major axis \( (u_x/u_y = 1:0) \) is shown in Fig. 7. The arrows in the figure indicate the loading direction. Birefringence retardation is denoted on a gray scale. Figure 7(a) shows the before-loading state. The part indicated by the white areas is considered to be the cell wall because of its shape; this part shows a good alignment of fibers. In contrast,
the part inside the boundary that is regarded as the cell wall shows small birefringence retardation. This shows that the birefringence inside the cell wall is smaller than that of the cell wall itself. The birefringence retardation distributions of the tissue aggregate after loading are shown in Figs. 7(b) and (c). These figures show an increase in the birefringence retardation of the cell wall and decrease in that inside the boundary. This result suggests that a remarkable alignment of fiber in the cell wall is brought about by loading stress in the direction of the fibers. On the other hand, the alignment inside the boundary is reduced by loading stress in a direction opposite to that of the original alignment.

3.4.2. Uniaxial tensile stress for minor axis

Figure 8 shows the birefringence retardation distribution when stress is loaded to the minor axis ($u_x : u_y = 0:1$). By comparing the birefringence retardation distribution before loading (Fig. 8(a)) with that after loading (Figs. 8(b) and (c)), it is noted that the birefringence retardation inside the boundary is increased by the loading of stress in contrast to that of the cell wall. If we assume that the alignment material exists inside the cell wall, it can be considered that the alignment of the material is increased by loading stress in the direction of the minor axis.

3.4.3. Biaxial tensile stress

The birefringence retardation distribution of a cell aggregate in the case of loading biaxial stress ($u_x : u_y = 1:1$) is shown in Fig. 9. Figure 9(a) shows the before-loading state. Figures 9 (b) and (c) show the after-loading state. Note that the birefringence retardation distribution did not change by loading stress on both the cell wall and its inner part. This result shows that though the sample consists of anisotropic cells, it has an appropriate structure to withstand the uniformly rising biaxial strain.

3.4.4. Correlation between macroscopic deformation and birefringence distribution

It is confirmed that the cell-level behavior differs with the loading strain direction as shown in Figs.7-9. The biaxial tensile test machine estimates only the displacement and cannot monitor the loading stress quantitatively. However, it was reported that the behavior of the macroscopic loading stress for an onion cell aggregate was different between the direction of the major and minor axis.$^8$

3.4.5. Possibility of birefringence observation

Birefringence is a vector with magnitude (retardation) and direction (azimuthal angle). Both the parameters are obtained by the foregoing birefringence measurement method as shown in Eq. (7). The correspondence between retardation and strain and that between azimuthal angle and strain loading direction were demonstrated using the biaxial tensile test machine for loading strain to a uniform sample.$^9$ However, only retardation distribution was adopted in this paper because a significant change in the azimuthal angle was not shown in loading strain to the onion cell. In order to examine the birefringence caused by loading stress more closely, both birefringence retardation and azimuthal angle need further consideration.

4. Conclusions

We developed a biaxial tensile test machine that can be installed in a birefringence microscope. The influence of the deformation of an individual cell on the macroscopic deformation of the cell aggregate is described with the observation of the birefringence of a plant tissue before and after applying tensile stress. The following conclusions were derived from the results and discussion.

(1) The effect of a change in strain distribution on the macroscopic deformation process...
Fig. 7 Phase difference distribution (major axis)

Fig. 8 Phase difference distribution (minor axis)
of a cell aggregate can be observed by a birefringence microscope.

(2) The small amount of birefringence caused by the deformation process of cells was visualized and quantified by employing the local-sampling phase shifting technique.

(3) The onion cell, taken as the sample plant tissue, shows differences in birefringence retardation distribution upon the application of tensile stress. From these results, the mechanical structure of a cell can be considered theoretically.

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