Measurement of thickness and profile of a transparent material using fluorescent stereo microscopy

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Abstract: Full-field thickness measurement for a thin transparent film is of interest for biological, medical, electronic, and packaging materials. It is a challenging task when the film is curvy, delicate and its thickness varies with location. We report herein a method to measure the thickness of a transparent (flat or curved) material and its topography using a stereo fluorescent profilometry technique. In this technique, two different types of fluorescent particles are deposited to both sides of the transparent film. Selected fluorescent excitation and emission are used to allow the observation of each one surface of the film at a time to determine the surface profile using stereo-based digital image correlation techniques. After the surface profiles for both surfaces are determined, subtraction of one surface profile from the other provides accurate thickness distribution of the film. Validation experiments were conducted using transparent films with known thickness. As an application, a measurement on a contact lens was conducted. The technique is appropriate for measurement of the full-field thickness of objects at other scales, such as soft transparent or translucent biofilms, with which thickness can hardly be measured accurately with other techniques.

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1. Introduction

Thickness of a thin film is an important geometric parameter that needs to be measured accurately in various situations, such as in the mechanical characterization of a tympanic membrane (TM) [1]. The available options for appropriate thickness measurement techniques are often times limited, especially when the thickness is small and the material is soft and delicate, or under moisturized conditions. In the case of a biofilm, or bio-membranes, the surface is curved, and the thickness varies with locations. Examples of such materials include TMs for human and animals (e.g., chinchilla, guinea pig), and cornea and lenses in eyes. The role of the thickness is important; an example is the assessment of retinal thickness, which is vital to understand the macular pathologies. It is very difficult to measure the location-dependent thickness of those curvy and delicate films. Existing techniques [2] for thickness measurement include micrometer, caliper, stylus profilometry, interferometry, reflectometry, ellipsometry, spectrophotometry, ultrasound, advanced light focused microscopy, laser scanning microscopy, optical coherence tomography (OCT) [3], ion beam analysis, X-ray reflextometry and tomography, electron microscopy and others. Some of these contacting methods are point-to-point measurement technique. Most of the existing thickness measurement techniques are used at the nanoscale for solid-state samples or relatively stiff samples. It remains a challenge for thickness measurement for a soft material at the microscale. Taking the full-field thickness measurement of the chinchilla eardrum as an example, the thickness is in the neighborhood of 20 μm, and varies with location. The thickness is out of capability of the ultrasonic method, which is in the range above 0.1 mm. Because eardrums are soft and delicate, a contact method is not appropriate. Instead, a non-contact method should be used. Non-contact methods, such as interferometry and reflectometry, have a capability for thickness measurement in the range of visible light wavelength. They are not suitable for samples with a thickness of tens of μm. A commercial OCT has resolution approximately 6 μm [4], it is not accurate enough for thickness measurement in many situations, such as measurement of TMs. Although OCT has been applied for measurement of the thickness of retinal, the standard deviation is on the order of 10 μm [5]. A sensitive method is needed to measure the thickness of a variety of films as described above. For a typical commercially available X-ray micro-computed topography, the highest resolution is approximately 2 μm/voxel [6–8]. In addition, a TM, like many other bio-tissues, is not X-ray sensitive, so that it is difficult to detect the surface edges of an eardrum for accurate thickness measurement. Laser scanning microscope will work for a sample cut from a TM, in which case the sample thickness can be different from the thickness of an intact TM [9]. In addition, it is very tedious, and as a result it is not possible to use it to
determine the thickness map of a TM. Other alternative methods such as scanning electron microscope (SEM), and transmission electron microscope, measure only thickness at edges in particular locations for a sample that is not in physiological condition. The thickness can be different from an intact full-size TM. Transmission electron microscope is suitable for very thin small samples, which are subject to very high-energy electronic beam bombarding, making it vulnerable to the beam damage. Currently, none method is available for non-contact, accurate, and full-field measurement of location-dependent thickness for a relatively soft, delicate and transparent material.

In this study, we report a new technique for full-field thickness measurement, and also the surface profile for a transparent, soft material at the microscale. The technique is based on a three-dimensional (3D) fluorescent stereo technique we developed for surface shape measurement at the microscale [10]. In the sequel, we describe the principal and the experimental setup for the technique, followed by validation and an example for application.

2. Principles and setup

The technique reported herein for thickness measurement for a transparent material with thickness on the order of microns or thicker is based on stereo digital image correlation of dual-path fluorescent images acquired for both surfaces of the film. This technique, referred to as stereo digital image correlation (also referred as 3D digital image correlation), is based on the principle of binocular stereo vision. It has been widely used in the research in experimental mechanics and materials science [11–13].

Figure 1 shows a schematic diagram for a binocular stereo-vision system. In a local Cartesian frame, a point (e.g., $P_1$) at the top surface of the film is projected into two points ($P_1^L$, $P_1^R$) in the imaging planes of the left and right cameras, respectively. Digital image correlation is used to find the corresponding points in the two images acquired by the two cameras. The binocular system is calibrated to determine parameters of cameras, so that the coordinates of the projected points in both images are obtained. Subsequently, the world coordinates for $P_1$ are reconstructed using triangulation method.

**Fig. 1.** A schematic diagram of the multi-fluorescent imaging system for thickness measurement on transparent materials.

In a previous study [10], we extended fluorescent digital image correlation for in-plane deformation measurement [14] to 3D profilometry and deformation measurements using the
fluorescent stereo microscopy based on stereo digital image correlation. The fluorescent particles are randomly sprayed onto the surface of a biofilm to form unique speckle pattern surrounding each point, and appropriate filters for excitation and emission are installed in the stereomicroscope.

In the biomedical imaging fields, tissues dyed with different fluorescent particles are observed using multi-path fluorescent imaging [15]. Two kinds of fluorescent particles are used to separate the top and the bottom surface. Dual-path fluorescent imaging provides an active technique to control the exciting of the particles. Thus, the dual-path fluorescent imaging technique is adopted for purpose of thickness measurement.

We refined a fluorescent stereo microscope (FSM) to allow the use of two fluorescent imaging paths for a transparent specimen. Two types of fluorescent particles are sprayed onto the top and bottom surfaces of a transparent curvy film as shown in Fig. 1. When turning on one excitation light, and the emission filter is rotated to match the excitation light, one particular layer of sprayed fluorescence is excited and the images are acquired by both left and right cameras. Using stereo digital image correlation, the 3D coordinates of the excited layer, representing geometry of one surface are reconstructed. Then the excitation layer for this particular surface is turned off. Subsequently the excitation light on the other surface is turned on, and the images are acquired by both cameras, and analyzed to determine surface topography of the other surface. With the coordinates for both top and bottom surfaces determined, by subtracting the 3D coordinates of the bottom surface from those for the bottom surface in Z-direction, the thickness distribution of the specimen can be obtained. For more complicated curvy transparent objects, the thickness can be determined using the coordinates in three directions accordingly.

Two appropriate fluorescent particles were selected in order to distinguish themselves under different excitation and emission wavelengths. Two types of fluorescent particles with diameter of 1 μm are chosen; they are blue-green fluorescent particles (430/465, Life Technology Corp., #F13080) and red fluorescent particles (580/605, Life Technology Corp., #F13083). Excitation and emission filters are used for fluorescent imaging. It is noted that the primary function of the emission/barrier filter in any fluorescent imaging system is to block the excitation wavelengths used and allow only the excited light from the fluorescent particles to pass. For the blue-green fluorescent, the matching filters are selected as the excitation filters EX420/40x and ET480/40m (Chroma Technology Corp.). For the other fluorescent particles, the matching filters are ET560/40x and ET615/40m.

Fig. 2. Band filters and properties of two types of fluorescent particles.
The properties of the band filters and fluorescent particles used in the setup are shown in Fig. 2. Blue and red are selected because the two colors are relatively far away from each other in the visible light spectrum. As shown in Fig. 2, the intensities associated with the two fluorescent imaging paths are clearly visible. The excitation band filter-1, and filter-2, and emission band filter-1, and filter-2 are used for the fluorescent imaging for fluorescent particle-1, and fluorescent particle-2, respectively.

Figure 3 shows the experimental setup, which is modified from a Zeiss OPMI stereo microscope to include dual fluorescent imaging paths. This microscope has a large working distance, up to 150 mm (the objective lens is 150 mm), and up to 5 mm depth of field. The viewing field is 5~18 mm in diameter, to allow measurements of relatively large surface profile and deformations. Two Nikon D7100 cameras are installed on the observation tubes of the stereomicroscope to acquire images of speckle patterns on either top or bottom surface of the sample. A beam splitter base is used to place the emission filters. A pair of aforementioned emission filters is placed under the base of the splitter base. Two different excitation filters (excitation band filter-1 and excitation band filter-2) are installed on the filter wheel on the white light illuminator Leica L5. When the fluorescent excitation filter is switched by rotating the filter wheel on the illumination, the pair of the emission filters can be removed and replaced with the other pair.

3. Experiments and application

A transparent glass slide is placed at the stage as shown in Fig. 3. The top surface of the sample is randomly sprayed with blue-green fluorescent particles and the bottom surface is sprayed with red fluorescent particles. The fluorescent random texture patterns on both surfaces are generated by an airbrush (Iwata Inc.). Before conducting the measuring experiment, it should take care to set the parameters for both cameras, such as exposure time, setting of ISO, and the intensity of illumination light should be adjusted for different fluorescent imaging paths, in order to eliminate the residual fluorescence effects under the unmatched fluorescent imaging paths. In this system, the residual fluorescent effect occurs as blue-green fluorescent particles (fluorescent particle-1) is excited when the red fluorescent imaging path (excitation band filter-2 and emission band filter-2) is used and vice versa. It is noted that it is important to detect the residual fluorescent effects in the system. For example, if the excitation band filter-2 and emission band filter-2 are used, the cameras should see...
nothing of a sample covered with only the blue-green fluorescent particles, and vice versa. If there is an effect on certain extent of residual fluorescent, the imaging parameters must be altered to eliminate the residual fluorescence effects. Figure 4 shows the fluorescent images of a sample acquired by the left and right cameras using the dual fluorescent imaging paths. The images have a resolution of 6000 × 4000 pixels. Figures 4(a) and 4(b) show the speckle patterns of the excited blue-green fluorescent particles using excitation band filter-1 and emission band filter-1. Then the excitation filter was changed to excitation filter-2 and the pair of emission filter-2 was replaced at the splitter modified base. Figures 4(c) and 4(d) show the speckle pattern of the excited red fluorescent particles using excitation band filter-2 and emission band filter-2. While the fluorescent paths are switched, the sample stays at the same position. The stereo microscope (with modified splitter base as shown in Fig. 3), provides an overall field of view of 8.22 × 5.48 mm with a resolution of 1.37 μm/pixel.

![Image](https://via.placeholder.com/150)

Fig. 4. Fluorescent images acquired by left and right cameras. (a)(b) blue-green fluorescent particles covering the top surface; (c)(d) red fluorescent covering the bottom surface.

It is seen clearly that the speckle patterns formed at the top and bottom surfaces are different. It shows that the two surfaces are distinguishable using the setup with dual-path fluorescent imaging. To obtain the thickness of the transparent sample, the same area of interest, as indicated by the yellow rectangle area, is viewed in Figs. 4(a) and 4(c).

If the X-Y plane is built on the glass slides, the Z-direction is in the thickness direction. After calibration of the system and stereo matching, the 3D coordinates of the top and bottom surfaces in the rectangle area can be reconstructed. Thus, the thickness is determined by subtracting the coordinates in Z-direction.

Additional experiments were conducted to examine the technique. A series of thin polyester films (McMaster, #8567k14, #8567k24 and 8567K44) with known thickness of one, two and four thousandth of inch (milliinch), were used. The top surface was sprayed with random blue-green fluorescent particles and the bottom surface was spared with red fluorescent particles. Two glass slides with thickness of 0.15 mm and 0.96 mm were also used for validation. Table 1 shows the data from experiments.

| Sample thickness                | Measured thickness |
|---------------------------------|--------------------|
| 1 milliinch (25.4 ± 2.54 μm)   | 23.8 ± 2.8 μm      |
| 2 milliinch (50.8 ± 5.08 μm)   | 48.2 ± 4.6 μm      |
| 4 milliinch (101.6 ± 10.2 μm)  | 97.6 ± 7.3 μm      |
| 150 ± 20 μm                    | 157.7 ± 13.2 μm    |
| 960 ± 20 μm                    | 893.3 ± 34.7 μm    |
Table 1 shows that the measured results are very close to the thicknesses of the samples except for the glass slide with a thickness of 0.96 mm. The large error on the glass slide of 0.96 mm thickness is due to the fact that while one surface is in focus, the other surface is not in focus. Once the speckle pattern on the surface is out-of-focus, it causes the decorrelation for the stereo-matching using digital image correlation. In our algorithm, the Newton-Raphson iterative method [13, 16] and reliability-guided digital image correlation [17] are employed to identify the corresponding points in matching images. It is noted that the accuracy of this thickness measurement technique should be at the same order as the displacement measurement of an object translated in Z-direction because the principles are the same [10]. The determination of the accuracy of the stereo digital image correlation could be very complex. Many factors play a role, these include quality of cameras, fluorescent particle size, speckle patterns, optical accessories, calibration, system configuration, stereo matching and algorithms [18, 19]. In this system, a modified base for the emission filters may affect the results. In addition, the fluorescent particle size is approximately 1 µm. The aggregate of the particle size can be even larger, so that the accuracy cannot be lower than the size of the aggregated fluorescent particle size. Furthermore, the residual fluorescent effects could affect the accuracy, despite that we have already set the exposure time and ISO setting carefully. However, there is room for further improvement in accuracy. For example, commercially available stereo digital image correlation system at the microscale, such as VIC-3D stereo microscope measurement system manufacture by Correlated Solutions, Inc [20], can achieve out-of-plane displacement resolution of ± 120 nm. Thus, the potential for improvement in accuracy is promising.

For application, we conducted thickness measurement on a contact lens. Contact lenses are transparent. The thickness distribution and surface shape are vitally important to achieve the desired optical properties. As an example for application of this technique, a measurement of the thickness of a contact lens (Bausch + Lomb, Daily disposable contact lens −4.00, diameter 14.2 mm) was conducted. The inner surface making contact with an eye was
covered with red fluorescent particles. The outer surface making contact with air is covered with blue-green yellow fluorescent particles. Figure 5 shows the red and blue fluorescent images, and the 3D reconstructed surfaces. The same area of interest was chosen as shown in Figs. 5(a) and 5(c). Using this technique, both surfaces of the contact lens are reconstructed. The full-field thickness is obtained as shown in Fig. 5(e). The average thickness in the central region is $76.0 \pm 6.6 \, \mu m$.

4. Conclusions

In conclusion, a new optical technique for measurement of full-field thickness and also surface topography of a transparent material was developed. In this technique, a combination of dual-path fluorescent imaging and stereo digital image correlation were used. Compared with other thickness measurement techniques, this technique is a non-contact full-field, and robust method for measurement of a soft transparent sample with complex and curvy surfaces. It is noted that in addition to measurement at the microscale, this technique is suitable for thickness and surface profile measurement at larger scales. With the use of stereo imaging at larger scales the method can be used for thickness measurement in the range of millimetres to meters.

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