Full-field transmission-type angle-deviation optical microscope with reflectivity-height transformation

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Abstract: This full-field transmission-type three-dimensional (3D) optical microscope is constructed based on the angle deviation method (ADM) and the algorithm of reflectivity-height transformation (RHT). The surface height is proportional to the deviation angle of light passing through the object. The angle deviation and surface height can be measured based on the reflectivity close to the critical angle using a parallelogram prism and two CCDs.

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1. Introduction

Chiu et al. [1] showed that the reflectivity-height transformation algorithm [2,3] could be used to determine a specimen’s surface height. The method was demonstrated for full-field three-dimensional surface profile measurements. A light from a small point of the test surface which is at the front focal plane is made to pass through the optical microscope and imaged at...
the back focal plane. The ray angle is found to be proportional to the gradient of the point. The system could be used for the characterization of internal reflection effect [4] near the critical angle in a prism to measure the reflectivity, thereby leading to the computation of the ray angle and the height of surface. This method differs greatly from traditional ones such as the White-Light Interferometry, the Optical Coherence Tomography (OCT) [5–7], Confocal Microscopy [8,9], NSOM: Near-field Scanning Optical Microscopy [10], and Laser-scanning Angle-deviation Microscopy (ADM) [11,12]. Among these reported methods, confocal microscopy [8,9] is best known as an optical scanning microscope. Its confocal system can be used to calculate the surface height through the measured defocal length. When the measuring point is not on the focal plane, the light intensity going through the pinhole on the receiving end will be reduced. This occurs because the pinhole can have a porous design which allows the lateral resolution of the image to overcome the optical diffraction limit. In NSOM [10], when obtaining a light intensity image through point-by-point scanning, the light intensity can differ due to the height or inclination of the measuring point. Since the signal is captured in smaller than a wavelength, it can overcome the diffraction limit and achieve nanometers of lateral resolution. The scanning type of ADM [11,12] combines surface plasmon resonance (SPR) wave with high-phase sensitivity and common-path heterodyne interferometry (CPHI) [13] with real-time and high-phase analytical characteristics. When the measuring point is out of the focal plane, the light beam will produce either divergence or convergence phenomenon. When this light beam is passed through an SPR prism, the rim of the beam will produce a phase change that is proportional to the height of the object being measured. Therefore, CPHI can be adopted to obtain the phase error between the two rim lights, while the surface topography can be obtained by using point-by-point scanning. Its lateral resolution is still influenced by the diffraction limit, but the axial resolution can achieve nanoscale accuracy.

Regarding non-scanning technology, either a low coherent light source or white light source can be used for interference microscopy to calculate the morphology of the test sample by analyzing the interference fringes. Most objective lenses are either Michelson, Mirau, or Linnik types [14]. A Mirau interferometer [15] has a design that creates nearly no optical path difference between the reference light and the test light and the whole structure is fully assembled on the objective lens. White light interference microscopes are ideal for capturing large-area images. This method is completely free of problems caused by interferometry environment disturbance and phase wrapping. Furthermore, in our proposed 3D ADM [2,3], no scanning occurs that leads to shortening of the measuring time. The samples are not damaged during the test and also do not require surface treatment in advance. Although lateral resolution is restricted by the optical diffraction limit, its performance regarding axial resolution is excellent, which could achieve nanoscale accuracy for the axial resolution. Moreover, this detection method has its unique advantages, such as relatively low cost and fast and requiring no scanning process.

In this paper, we propose a full-field transmission-type 3D ADM with a magnification from 50X to 120X for transparent materials or biological sample measurements without scanning. We will analyze its measurement errors including the system errors and the operational errors. The system errors include the errors caused by the system’s optical components, light source, and angle deflection direction. The operational error is the main error due to the object location or the CCD’s position relative to the focal plane. This work is similar to the reflection-type ADM [3, 17] but the structure is different. This transmission-type ADM with the magnification factor spanning from 50X to 120X is larger than the designs in the previously reported works [2, 3] and the calculation method decreases the nonlinear error. The error analysis is also presented in greater details in this paper.

The method and geometric structure adopted in this paper are very simple. The system’s construction, alignment and calibration can be easily carried out for the proposed system would result in accurate lateral and axial measurements to reach sub-micron and nanometer levels, respectively. The mainly transparent object can only be measured when sufficient light intensity is present. The surface of the object will not be damaged, as the process is just like taking a photo, and the 2D and 3D surface profiles of the object can be measured in real time.
2. Principles

2.1. Angular sensor

A parallelogram prism is used as the angular sensor in order to measure its reflectivity of the secondary internal reflection, as well as to obtain the angle of incidence. Because the height difference between two adjacent points is in direct proportion to its light angle deviation \( \Delta \theta \) (Please refer to Section 2.2), the deviation angle can be calculated with this reflectivity difference value, after which the surface height difference can be obtained, followed by the surface profile of the object.

![Light propagation with a small angle deviation in a parallelogram prism.](image)

A light propagation in a parallelogram prism is shown in Fig. 1. We take s-polarization as an example, and its reflectivity of twice internal reflection based on Fresnel's law can be written as:

\[
R_s^2 = R_y^2 = \left( \frac{n_1 \cos \theta_1 - n_2 \cos \theta_2}{n_1 \cos \theta_1 + n_2 \cos \theta_2} \right)^2,
\]

where, \( R_s \) and \( R_y \) are the reflectivity of twice and once internal reflections; \( n_1 \) and \( n_2 \) are the indices of refraction of the prism and air, respectively; \( \theta_1 \) and \( \theta_2 \) are the angles of incidence and refraction on the interfaces of hypotenuse, respectively, and \( \theta \) is the angle of incidence at entrance face. If the light has a small angle deviation \( \Delta \theta \) and the incident angle is within the region of \( \theta > \theta_c \), where \( \theta_c \) is the critical angle at entrance face, the reflectivity \( R_s \) will be changed. The light trace change due to angle deviation \( \Delta \theta \) is shown in Fig. 1. \( R_s \) only considers the twice internal reflections and does not include the intensity changes of other faces. The power of the outgoing light is normalized and the actual measuring result of the parallelogram prism reflectivity is shown in Fig. 2(a), which has its angle rotated along with the rotation stage. The rotation range of the external angle is from 0° to 10°, during which the power meter will perform continuous measurements. If the prism is made of BK7 and placed in air, then \( n_1 = 1.51509 \) and \( n_2 = 1.0003 \) for the wavelength of 632.8 nm. Therefore, the critical angle of external incident angle is 5.61° (i.e. \( \theta = 5.61^\circ \)) and the angle of TIR is smaller than 5.61°.
Transforming the range of coordinates of $\theta > 5.61^\circ$, $R_{s2}$ can be taken as the x-axis, and $\theta$ as the y-axis, so that the reflectivity $R_{s2}$ can be measured, and $\theta$ as $R_{s2}$’s function; $\theta(R_{s2})$ can be expressed as:

$$
\theta(R_{s2}) = 17R_{s2}^7 + 21R_{s2}^6 - 2.3 \times 10^2 R_{s2}^5 \\
+ 4.2 \times 10^2 R_{s2}^4 - 3.7 \times 10^2 R_{s2}^3 + 1.7 \times 10^2 R_{s2}^2 - 45R_{s2} + 12
$$

The transformed curve and the fitting curve are shown in Fig. 2 (b). The measured $R_{s2}$ is inserted into Eq. (2) to obtain $\theta$. In addition, the change of $R_{s2}$ is measured and the angle deviation $\Delta \theta$ is calculated.

2.2. Reflectivity-height transformation

Figure 3 is the schematic diagram of the light which is normally incident into the test sample. The indices of refraction of the sample and air are $n_1$ and $n_2$, respectively. If the test surface in air has an angle of inclination $\alpha$, and the emergent light’s deviation angle is $\beta$, then $\alpha$ can be written as

$$
\alpha = \frac{\beta}{n_1 - n_2}.
$$

According to the trigonometric relationship, when the space between two points is $\Delta x$, let $\Delta n = n_3 - n_2$, then the height difference between the two points is

$$
\Delta h = \alpha \Delta x = \frac{\beta}{\Delta n} \Delta x.
$$
If the light is incident on the microscope and the measuring system, and when the microscope’s magnification is $M$, then the deviation angle of the microscope’s emergent light is $\beta / M$. The angle deviation at the entrance face of the parallelogram prism is $\Delta \theta = \beta / M$, which will cause a change in reflectivity, namely its variable quantity is $\Delta R_{\alpha, \beta}$. After obtaining from Eq. (4), $\Delta h$ can be written as

$$\Delta h = \frac{M \Delta \theta}{\Delta n} \Delta x. \quad (5)$$

If the value of the pixel size $\Delta X$ of the known CCD is a constant, then the space between two points relative to the test surface is $\Delta x = \Delta X / M$; therefore, Eq. (5) can be rewritten as

$$\Delta h = \frac{\Delta X}{\Delta n} \Delta \theta = A \Delta \theta, \quad (6)$$

where, constant $A = \Delta X / \Delta n$, and $\Delta \theta$ is small, then the height of each point is

$$h(x_i, y_j) = \sum_{m=1}^{i} \Delta h_{m,j} + h_0 \quad (7)$$

Assuming that the original height is $h_0$, and $\Delta h_{i,j}$ is height difference between $h(x_i, y_j)$ and $h(x_{i-1}, y_j)$, the height of each point in each line on the CCD can be figured out according to Eq. (7). Finally, we can draw the 3D surface profile of the measured object.

3. Experimental results

The experimental setup is shown in Fig. 4. A light from a He-Ne laser (1) passes through an isolator (polarizer (2) & quarter wave plate (3)) to prevent the reflected light of the optical system from returning to the laser inside. It is expanded into a parallel beam by a beam expander (4) and then obtains vertical polarization (s-polarization) after passing through a polarizer (P(90°), 5) whose transmission axis is at 90°. An iris (6) is used to limit the size of the expanded beam. An afocal imaging system (including an objective (8a) and a lens (8b)) magnifies the image after passing through the sample (7). The light is divided into transmission light and reflected light when passing through a beam-splitting prism (9); The transmission light reflects twice in a parallelogram prism (11). The rotation angle near the critical angle is controlled by a controller (Newport: ESP-300, 14) by a combination of a rotation stage (15). CCD1 (13) captures a critical angle image (as shown in Fig. 2(a), $\theta > 5.6$) whose intensity is $I_1(X,Y)$. The reflected light reflects once through a right-angle prism (10). CCD2 (12) captures the total-internal reflection (TIR) image whose intensity is $I_2(X,Y)$. Every CCD is directly connected to a computer (16) by USB for software display of image shots.
The sample’s three-dimensional surface profile is finally described by the image overlapping and reflectivity calculation \( R_{s2}(X,Y) = I_1 / I_2 \) using the Matlab software. The results are put into Eq. (2) to calculate the matrix of the angle \( \theta \) and \( \Delta \theta \), and then substituting \( \Delta \theta \) into Eq. (7) to obtain the height of each point and finally draw the specimen’s 3D surface profile.

3.1. Effect from the angle deflection direction

This experiment aims to examine the effect on the angle deflections of the object light. For proving the results in the orthogonal directions of angle deflection to be the same, this experiment is carried out by rotating the object being tested instead of rotating the prism for the convenience of operation, namely holding the parallelogram prism to lay flat. As a result, before rotating the object, the s-polarization of test light will immediately lead to a change in reflectivity after passing through the parallelogram prism (PP, 11). This angle deflection direction is on the parallel plane(x-z plane). After rotating the object by 90°, it is equivalent to the orthogonal direction of the angle deflection of the object light, which experiences another reflectivity reaction after passing through the parallelogram prism (PP, 11). The following discusses the difference between the two mutually orthogonal angular deflections on the measurement of surface profile based on the experimental results. The steps are as follows: As shown in Fig. 4, the magnification of microscope is 120X. First, the specimen is placed onto the front focal plane of the objective (8a) and the laser light has normal incidence upon the surface being tested. According to the above practice to capture the critical angle image \( I_1(X,Y) \) and TIR image \( I_2(X,Y) \), the reflectivity matrix \( R_{s2}(X,Y) = I_1 / I_2 \) is calculated and then placed into Eq. (2) to calculate the angle matrix \( \theta(X,Y) \). The matrix \( \Delta \theta \) of the angle deviation from \( \theta(X,Y) \) is obtained. \( \Delta h(X,Y) \) is then calculated and substituted into Eq. (7) to obtain height \( h \). Next, the object being tested is rotated 90°, and the above steps are repeated to calculate the height \( h \). Finally, the measured heights before and after the rotation are compared.

The known height of 80 lines/mm grating which is a transmission type grating made of glass \( (n_g = 1.5) \) and carved by a machine is taken as the sample to perform the measurement at \( \theta = 5.9° \), with its 3D profile measurement results before and after rotation shown in Figs. 5(a) and 5(b). Figure 5(b) rotates the result 90° in the reverse direction to make it the same direction as in Fig. 5(a) for comparison and observation. Both surface profile and height are quite similar and there is little difference between the surface profiles of the orthogonal angle deflection directions on the x-z plane and y-z plane. Figure 5(c) shows a 2D profile that illustrates that the height presented by the grating before the 90° rotation almost perfectly matches the height after the rotation. The average heights before and after rotation are 150.4
nm and 151.5 nm, respectively. The experimental results are compared with that of the AFM (as shown in Fig. 5(d), where the average height is 153.5 nm). Using AFM results as a reference, we determine that their percentage errors are 2.02% and 1.30%, respectively, with the relative error between them being 0.7%.

This small difference shows that the 3D profile of the surface being measured can be drawn in any direction of angle deflection at the $\theta = 5.9^\circ$. The result is similar to the result of reflection-type 3D ADM [17].

The other specimen is Euglena. Whose 3D images before and after the 90° rotation are shown in Figs. 6(a) and 6(b), respectively. The external incident angle is at 8.5° and $\Delta n = 0.37$. For getting easy comparison and observation of these two results, Fig. 6(b) shows the same direction as Fig. 6(a), the actual image that was rotated counterclockwise 90°. Their average heights are 794.4 nm and 813.2 nm, respectively, with errors for both being 18.8 nm. The results also showed that the height error before and after rotation is very small, with a 2.3% relative percentage error. When the external incident angle increases, the percentage error increases.
4. Discussions

4.1. Effect of image blur

When the depth of the field of the objective is 0.8 μm and the optical magnification is 120X, the depth of focus of the image is found to be 11.52 mm. Therefore, the maximum departure from the focal plane is 5.76 mm, or the image is blurry. In the worst scenario in the eye’s judgment, just let the CCD position from the clearest point to be +5.76 mm or −5.76 mm.

In Fig. 7, the solid line, the dashes, and the dotted line represent the results of the 2D surface profiles of a grating with 80 lines /mm when the CCD is located on the image plane and departing 5.76 mm forward and backward from the plane, respectively. Table 1 shows

|                | CCD at the clearest image plane | CCD with forward displacement of 5.76 mm | CCD with backward displacement of 5.76 mm | AFM |
|----------------|---------------------------------|------------------------------------------|-------------------------------------------|-----|
| Average height (nm) | 150.3                           | 158.7                                    | 158.3                                     | 153.5|
| Average percentage error | 2.04%                           | 3.41%                                    | 3.13%                                     |     |
that the maximum and minimum percentage errors of the three cases compared with that of
the AFM are 2.04% and 3.41%, respectively. Thus, including the 0.7% of deflection direction
effect, the maximum percentage error is about 3.5% with respect to AFM’s result.

4.2 System sensitivity

Sensitivity ($S$) is defined with the partial differential equation below:

$$S = \left| \frac{\partial R}{\partial h} \right|$$

(8)

If the measuring scope of the angle of incidence is 5.8°~10°, then the best sensitivity is
0.041 (change/nm), which occurs when the external angle is 5.8°. The worst sensitivity is
1.563 × 10^{-4} (change/nm) which occurs when the external angle is 10° for $\Delta n = 0.5$. The
sensitivity curves of different values of $\Delta n$ are shown in Fig. 8, where the solid line, the
dashes, and the dotted line represent the results of $\Delta n = 0.5, 0.3, 0.1$, respectively. Therefore,
the sensitivity of height descents as the incident angle increases.

![Sensitivity curves](image)

Fig. 8. The height sensitivities at different incident angles for different sample of $\Delta n = 0.1,$
0.3 and 0.5.

4.3. Axial resolution

In axial resolution $\Delta h_{\text{min}}$, $S$ is sensitivity and $\Delta R_{S2}$ (min) is the CCD’s identified minimum
reflectivity variation, with its CCD in gray-scale value being 0~255, namely the change value
of 8bit. When $\Delta R_{S2}$ (min) = 1/256, the light intensity’s instantaneous variables are all lower
than 0.1%, and because they are less than 1/256, the error triggered by light intensity can be
ignored. Sensitivity $S$ is defined as the reaction of reflectivity to minimum height variation,
and axial resolution $\Delta h_{\text{min}}$ can be written as:

$$\Delta h_{\text{min}} = \frac{\Delta R_{S2}}{S}$$

(9)

The ideal axial resolution is ± 2 nm when $\theta = 5.9°$. However, from repetitive
measurements, we found that the actual axial resolution is ± 3 nm. This result was found
because the maximum light intensity is not just 255 and the darkest light intensity is not just
0; therefore, 1/256 can only be obtained under ideal conditions. Furthermore, we consider
whether there is recorded time difference between the critical angle image and TIR image
where the light source’s flicker problem cannot be eliminated.

The axial resolution under this system is shown in Fig. 9 for different refractive index with
$\Delta n = 0.1$~0.5. When $\theta = 7°$, the ideal axial resolution is 5 nm and when $\theta = 8°$, the ideal
axial resolution is 10 nm for $\Delta n = 0.5$. From these curves, the axial resolution decreases as
the incident angle increases or/and the $\Delta n$ decreases.
4.4 Lateral resolution

The CCD’s size is 1024 × 768 (pixel × pixel) and the pixel size is 4.65 μm × 4.65 μm for the CCD shown in this paper. The NA value of the used objective is 0.85. The lateral resolution is the resolving power and its equation is as follows:

\[ y_m = \frac{0.61 \times \lambda}{NA} \]  

(10)

Therefore, the lateral resolution \( y_m \) toward the x-direction and y-direction is 0.45 μm for a grating of 80 lines/mm and other samples.

4.5. Measurement range

Equation (6) explains that the height change is directly proportional to the angle change. In this experiment, the angle measuring range is 5.8~8.0 degrees, \( \Delta n = 0.5 \), \( NA = 0.85 \), depth of field is 0.8 μm the light with a wavelength of 0.6328 μm, and \( M = 120X \). The lateral resolution is 0.454 μm and the image size of the interval is equal to 12 pixels for the pixel size of 4.65 μm × 4.65 μm. According to the theory of Resolving Power, the measurable height range between two adjacent points with the interval of 0.454 μm is 0.37μm~4.65 μm in theoretical calculations. But the depth of field is still only 0.8 μm. Therefore, the real maximum measurement range of the height for the angle of incidence of 5.8–8.0 degrees is 0.8 μm. For \( NA \) and magnification, if \( NA = 0.85 \), \( M = 120 \) times, the angle deviation is limited within ± 58.2 degrees and the maximum angle deviation in the image plane is ± 0.49 degrees.

From Eqs. (6) and (7), the height difference \( \Delta h \) is ± 0.954 μm and the theoretical maximum height is within 6.79 μm for CCD’s size of 1024 × 768 (pixel × pixel), \( \Delta X = 12 \times 4.65 \) μm, and \( \Delta n = 0.5 \). This value is also larger than the depth of field (0.8 μm). Therefore, for this experimental structure, the height measurement range is ≤ 0.8 μm.

4.6. Summary

Although the available maximum deviation angle is 0.49° in image plane in this case, \( NA = 0.85 \), the range of deviation angle is limited by the critical angle when \( \theta \leq 6.1^\circ \). For example, if \( \theta = 5.9^\circ \), \( \Delta n = 0.5 \), its maximum deviation angle is 0.29° because the critical angle \( \theta = 5.61^\circ \). The measurement range of the height between two points under the lateral resolution 0.454 μm is below 565 nm. Its axial resolution is ± 3 nm and the minimum percentage error is about 0.5%. If the height is 85nm, the percentage error is 3.5%. If \( \theta > 6.1^\circ \), the available deviation angle in image plane is limited by the value of \( NA = 0.85 \) is 0.49°, and the maximum height range is limited by depth of field (0.8 μm). For example, \( \theta = 8.0^\circ \), \( \Delta n = 0.5 \), the axial resolution is 10 nm, and the measurement range of height between two points under the lateral resolution 0.454 μm is below 800 nm. Thus the minimum
percentage error is 1.3%. If the height is 200nm, the percentage error is 5.0%. Therefore, the percentage error is influenced by the incident angle, sample height, lateral resolution, $NA$, depth of field and magnification. The available measurement range of the height is decided by depth of field, $NA$, and incident angle. The other error induced by the depth of focus (the image blur effect) is within several percentages.

4. Conclusion

The feasibility of a transmission-type 3D ADM is demonstrated. The main error is from the image blur and incorrect selection of incident angle. The effect on the direction of angle deflection of object light can be ignored. The system percentage error is 3.5% compared with the results of the AFM when $\theta = 5.9^\circ$. The best lateral and axial resolutions are respectively 0.454 $\mu$m and 3 nm in this experimental setup. Therefore, this method would be suitable and acceptable for many applications. The main advantages are its relatively inexpensive structure and high speed capture because it is without any scanning component or drive and the capture time is only decided by the software and the speed of CCD.

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