Full-field Three-dimensional Angle-deviation Optical Microscope

Ming-Hung Chiu¹, Chen-Tai Tan¹, Ming-Hung Tsai¹, Ya-Hsin Yang¹
¹Department of Electro-Optical Engineering, National Formosa University, Huwei, Yunlin, Taiwan, ROC
* Corresponding Author / E-mail: mhchiu@nfu.edu.tw, TEL: +886-5-6319666, FAX: +886-5-6329257

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This study proposes a full-field three-dimensional (3D) optical microscope based on reflectivity-height transformation. The slight angle deviation of the test light is caused by the sample’s surface gradient; meanwhile, the light that normally passes through the sample is sensed by a parallelogram prism and transformed into reflectivity. Therefore, the reflectivity of the prism near the critical angle is proportional to the height of the surface. Using this method for microscopy has such merits as full field imaging without scanning, high vertical resolution, a simple and inexpensive structure, and real-time measurements. The errors benchmarked using the experimental results from the Atomic Force Microscope (AFM) are less than 4%.

1. Introduction

The greatest characteristic of three-dimensional (3D) optical microscopy is that invasive examination of a test sample is not necessary. Other 3D techniques, such as the stylus profiling method [1], the scanning probe microscope (SPM) [2], or the scanning electronic microscope (SEM) [3], can destroy a sample’s surface during measurements. The stylus scanning microscope has such characteristics as simple operation with a slow scanning speed, small tip radius with a large pressure borne by the measured surface, the ability to easily damage soft samples, and the potential to cause probe damage on hard samples, thus making it clearly inappropriate for the measurement of soft surface contours. On the other hand, optical microscopy has such advantages as a low cost, simple operation and almost no preparation procedure for test samples, in addition to taking non-destructive and non-contact measurements. Furthermore, test samples can be placed directly on the platform during observation. Measurements are faster in optical microscopy than in stylus scanning microscope, but its unique disadvantage is that lateral resolution is difficult to increase because of the restriction of the diffraction limit.

Garini et al. [4] categorized optical microscopes into two-dimensional (2D) and three-dimensional (3D) measurement methods. The 2D surface measurement method can avoid the occurrence of diffraction and interference, overcome diffraction limits and increase resolution to tens of nanometers during near-field observation of samples of NSOM: Near-field Scanning Optical Microscopes [5] (with a measurement distance of less than one wavelength). TIRFM: Total-internal Reflection Fluorescent Microscopy [6] will generate a kind of decay wave whose energy decays exponentially and triggers only tens of nanometers of fluorescent molecules on the interface to reach nanometer level resolution. SPR: Surface Plasmon Resonance [7] utilizes the high phase sensitivity of the exponential decaying field of the triggered surface Plasmon wave at the resonance angle on the interface between metal film and the test medium. A prism coated with a metal film serves as a sensor for surface profile measurements.

Three-dimensional measurement methods are also categorized into conventional microscopy [8], confocal scanning microscopy [9-10], interference microscopy [11-15], and multiphoton microscopy [16]. Confocal scanning microscopes have one more pinhole than conventional microscopes, which is on the back focal plane of the collecting lens. The partial light reflected from the test surface can pass through the pinholes when the specimen’s surface is on the front focal plane of an object. In other words, most of the energy is blocked by the pinholes if the sample is unfocused. In comparison with a conventional microscope, the optical diffraction limit can be broken and the lateral resolution will be better. However, the axial resolution still depends on the value of the numerical aperture (NA).

Regarding interference microscopy, interference objectives are most commonly Michelson, Mirau [11], or Linnik types [12]. The Mirau interferometer uses a very compact optical system that can be incorporated in a microscope objective. Applying white-light interferometry [13-15] to scan the object in depth can overcome the problem of 2π phase ambiguities.

In the non-linear method, the measurement scope of multiphoton microscopy (MPM) [16] can reach a depth of 500 μm, such as a mouse’s ovary cell, without damaging the cells or organisms around the biological sample.

Chiu et al. proposed the reflection type [17] and transmission type...
We proposed a large-area non-scanning intensity method for a full-field angle-deviation profilometer in 2011 [20-21]. This method differs from all other reported microscopy methods in that it utilizes a parallelogram prism as an angular sensor and measures reflectivity with a combination of CCD image capturing technology at the critical angle. The reflectivity patterns are achieved by overlapping two images (the critical angle and the total internal reflection (TIR) images) point by point and carrying out the intensity division (the ratio of the intensity at the critical angle to the intensity at the TIR). The reflectivity on each pixel represents an incident angle; thus, the angle deviation can be represented by the gradient of reflectivity and directly converted to the surface height difference between two adjacent points of the test surface. Then the angle deviation of every point is calculated and converted into a surface profile using the triangular geometric relationship. We measure transmission type gratings of a depth of 80 nm by the p- and s-polarizations, respectively. The results show that sensitivity can achieve 8.22×10⁻³ (change/nm) when using p-polarized light. Of the small measurable longitudinal height scope, the best axial resolution is 0.5 nm while the best lateral resolution is about 1 μm. Sensitivity is only 4.7×10⁻³ (change/nm) when measuring the sample with the s-polarized light. Of the larger measurable longitudinal height scope, the best lateral resolution is 0.42 μm and the best axial resolution is 0.7 nm. The error compared with other instruments can be reduced to 3.23% after nonlinear error compensation. The height measurement scope is from tens of nanometers to several microns.

As shown in Table 1, this method is superior to other commercial microscopes from the perspectives of operation environment, sample preparation, imaging size, real-time measurement, destructivity and contact, with an axial resolution that can achieve 0.7 nm. Although the lateral resolution is restricted by the diffraction limit, further improvement may be possible with future research.

![Table 1 Comparison of various microscopies](image)

2. Principles

2.1 Structure of the full-field transmission-type three-dimensional ADM

Fig. 1 shows the experimental setup used in this study. 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 sensor to measure the sample’s surface profile.

We measure reflectivity to obtain the incident angle and transform it into surface height. The third dimension of an object can be derived directly from CCD’s images. Using a confocal system as the imaging system for the final imaging on the back focal plane of the second lens, the same sample is measured with the s- and p-polarizations, respectively, to compare the results’ differences.

The optical magnification of this system is 50–120X if using objectives whose NA value is 0.9. We measure the known groove depth of a grating of 40 lines / mm for system calibration and error estimation and then compare those with the measuring results of other instruments to demonstrate the feasibility of our structure and idea.

The results show that this method can successfully measure the full-field 3D surface profiles of samples, such as polystyrene, Euglena, etc. The best lateral resolution is 0.42 μm and the best axial resolution is 0.7 nm. The error compared with other instruments can be reduced to 3.23% after nonlinear error compensation. The height measurement scope is from tens of nanometers to several microns.

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system from returning to the laser inside. Said light is expanded into a parallel beam by a beam expander (4), and then obtains vertical polarization (the s-polarization) after passing through a polarizer (P(90°), 5) whose transmission axis is at 90°. An iris (6) is used to adjust the expanded beam size, and the microscope’s confocal system (including an objective and a lens, 8) magnifies the image of the sample (7). Then the light is divided into transmission light and reflected light when passed through a beam-splitter (BS) (9); the transmission light is reflected twice in a parallelogram prism (11). The angle of incidence near the critical angle is adjusted to a specific angle by a controller (Newport: ESP-300, 14) through a combination of a rotation stages (15). CCD1 (13) captures the critical angle image. The reflected light from BS (9) is totally reflected once through a right-angle prism (10). CCD2 (12) captures the total-internal reflection (TIR) image. Every CCD is directly connected to a computer (16) by USB for a software display of the images. The sample’s three-dimensional surface profile is finally described by the image overlapping and reflectivity calculation performed by the MATLAB software.

Fig. 1 Experimental setup. 1= laser; 2= polarizer; 3= quarter wave plate; 4= beam expander; 5= polarizer; 6= iris; 7= sample; 8= confocal system with an objective and a lens; 9= beam splitter (BS); 10= right angle prism (RP); 11= parallelogram prism (PP); 12= CCD2; 13= CCD1; 14= rotation stage controller (Newport: ESP-300); 15= rotation stage; 16= personal computer (PC).

2.2 Reflectivity-height transformation

Fig. 2 shows the light incident striking a transparent flat plate with a slight deviation angle, in which the apex angle is \( \alpha \), the incident angle on the 1st interface is \( \theta_1 \), the outgoing angle on the 2nd interface is \( \theta_2 \) and the deflection angle is \( \beta \). The deflection angle can be calculated by using Eq. (1) according to geometric optical theory.

\[
\beta = \theta_1 + \theta_2 - \alpha
\]  

From Fig. 3, we can see that, in normal incidence, \( \theta_1 = 0 \), and the test light falls onto the sample according to paraxial optics approximation theory when \( \alpha \) is very small. The refractive index of the plate is \( n \). From Equation (1), the angle \( \alpha \) can be given by

\[
\alpha = \frac{\beta}{n - 1}
\]  

The transmission light will not create angle deviation when \( \alpha = 0 \). In other words, from Fig. 3, the small height difference \( dh \) is proportional to the apex angle \( \alpha \) and can be expressed as follows:

\[
dh = \beta dx = \frac{\beta}{n - 1} dx
\]  

Fig. 2 Angular deviation of a beam incident into a transparent specimen

Fig. 3 A light deflects a small angle \( \beta \) from its original direction after passing through a transparent sample with a small apex \( \alpha \)
As shown in Fig. 4, an objective and a lens constitute the confocal system for the final imaging of the back focal plane on CCD. An ABCD matrix is utilized to calculate the height of ray ($r_1$) and the ray angle ($r'_1$) of every point of the image shown in Eqs. (4) and (5), respectively.

$$r_1 = \frac{f_2}{f_1} x_s = M \cdot x_s$$  \hspace{1cm} (4) \\
$$r'_1 = \frac{f_1}{f_2} x'_s = \frac{x'_s}{M}$$  \hspace{1cm} (5)

Meanwhile, the optical magnification of the imaging system is calculated, namely, $M = \frac{f_2}{f_1}$. An interval on the image can be written as $dX = Mdx$. That is, it is directly proportional to the absolute value of the specific ratio of the focal length of the lens system. The variation of the angle of incidence is the deflective angle divided by optical magnification, i.e. $d\theta = r'_1 = \frac{x'_s}{M} = \beta / M$.

Fig. 5(a) displays the light with the s- or p-polarization and incident into a parallelogram prism for double internal reflection. Eqs. (6) and (7) are the reflectivity of double internal reflection in the s- and p-polarizations, respectively.

$$R_{s2} = \sqrt{R_s}^2 = |R_s|^4 = \left| \frac{n_1 \cos \theta_1 - n_2 \cos \theta_2}{n_1 \cos \theta_1 + n_2 \cos \theta_2} \right|^4$$  \hspace{1cm} (6) \\
$$R_{p2} = \sqrt{R_p}^2 = |R_p|^4 = \left| \frac{n_2 \cos \theta_1 - n_1 \cos \theta_2}{n_2 \cos \theta_1 + n_1 \cos \theta_2} \right|^4$$  \hspace{1cm} (7)

The s-polarization descends slowly while the p-polarization descends rapidly when the incident angle is larger than the critical angle, i.e. $\theta > 56.1^\circ$. The curves in Fig. 5(b) show that the gradients of these two curves with different descending trends clearly differ. Utilizing the comparison of the gradient characteristics of the s- and p-polarizations, the same sample can be measured and the difference compared. From Eqs. (8) and (9), we can substitute $d\theta = dR_{s2} / m_s = dR_{p2} / m_p$, $\beta = M d\theta$ and $dx = dX / M$ into Eq. (3) to deduce the surface height change equations of the s- and p-polarizations, namely, Eqs. (10) and (11), respectively.

$$dh_s = \frac{dR_{s2}}{n-1} m_s dX$$  \hspace{1cm} (10) \\
$$dh_p = \frac{dR_{p2}}{n-1} m_p dX$$  \hspace{1cm} (11)

2.3 Nonlinear error compensation

The surface height change is proportional to the variation of reflectivity. However, in real cases, the angle deviation and gradient ($m_s$ or $m_p$) vary with surface height. That is, the conversion of reflectivity to height is not a linear function. If we regard the gradient of all points within a larger range of height measurement as both average values and fixed values [22], the height calculations will cause nonlinear errors and distortion. Therefore, $m_s$ and $m_p$ should be regarded as variables. Considering $\frac{dX}{n-1} = A_s$ as a constant and $\frac{dX}{n-1} = A_p$ as another constant, i.e. $A_s = A_p = \frac{dX}{n-1} = A$ and by substituting the value $A$ into Eqs. (10) and (11), those
Equations can be rewritten as
\[ dh_s = A \frac{dR_{s2}}{d\theta} = Ad\theta \]
and
\[ dh_p = Ad\theta. \]  

As previously mentioned, the angle is measured to obtain the surface profile and compensate the original nonlinear error to achieve more correct and accurate measurements. Therefore, we combine Eqs. (14) and (15) to obtain the surface profile of the s- and p-polarizations shown in Eqs. (16) and (17), in which \( h_0 \) refers to initial height while the initial angle of incidence is at \( \theta = \theta_0 \), namely, \( h_0 = A\theta_0 \).

\[ h_s = A_s \int d\theta = A\theta + h_0 \]  
\[ h_p = A_p \int d\theta = A\theta + h_0 \]

2.4 The measurement scores in the s- and p-polarizations

The array size of CCD is 1024×768. Taking the s-polarization as an example, \( R_{s2} \) corresponds to \( \theta \) for every array element and, thus, the array dimension of \( \theta \) is also 1024×768. From Fig. 6(a) and the \( R_{s2} \) curve equation, we can see that calculating \( \theta \) (X-axle) with the \( R_{s2} \) (Y-axis) measured by CCDs is difficult. We can convert the coordinates of \( R_{s2} \) and \( \theta \) shown in Fig. 6(a) to obtain Fig. 7(a), namely, the conversion of \( \theta \) into the Y-axis and \( R_{s2} \) into the X-axis.

By substituting \( R_{s2} \) matrix elements into the X item to easily calculate \( \theta \) matrix elements of the Y-axis, we can also calculate the surface profile after considering the gradient a variable for correction. Likewise, for the p-polarization, the coordinates of Fig. 6(b), \( R_{p2} \) versus \( \theta \), are exchanged as shown in Fig. 7(b).

From Eqs. (16) and (17), although similar, Figs. 7(a) and 7(b) show that the angle functions \( \theta(R_{s2}) \) and \( \theta(R_{p2}) \) are different. Fig. 7(a), the coordinate conversion figure of \( R_{s2} \) and \( \theta \), shows that the \( R_{s2} \) value of the actual curve almost overlaps with that of the fitted curve, proving that the gradient of reflectivity of the s-polarization to a larger external angle of incidence is small and of low angle sensitivity for a larger scope of height measurement (larger than 600 nm). On the other hand, Fig. 7(b), the coordinate conversion figure of \( R_{p2} \) and \( \theta \), shows that the actual curve only overlaps slightly with the left part of the fitted curve because the curve deviation increases rightward. We can calculate that the measurement will become incorrect if \( R_{p2} \) increases, and vice versa. Therefore, the curve of reflectivity of the p-polarization to an external angle of incidence is steep and of high angle sensitivity, suitable for measurements within a scope of small heights (less than 100 nm in experience), but unsuitable for measurements within a scope of large heights (more than 100 nm).

3. Experimental results and discussions

3.1 Experimental results of nonlinear error compensation

The structure of the test is shown in Fig. 1, in which a He-Ne laser (1) with a wavelength of 632.8 nm is used as a light source and the...
sample grating is 20 lines/mm (Focal length of an objective of a confocal system of \( f_1 = 2.54 \) cm; \( N.A = 0.25; \) the second lens of \( f_2 = 30 \) cm; \( M = -12.7; \) Measurement by the s- and p-polarizations, respectively).

1) In the s-polarization

After nonlinear error compensation in the s-polarization of the 2D surface profile of the grating as shown in Fig. 8(b), the average height is 79.4 nm. By comparing Fig. 8(b) with the uncorrected results shown in Fig. 8(a), the profile dithering amplitudes of the grooves are clearly reduced because of using the actual fitting curve in Fig. 7(a) to carry out the reflectivity-height transformation in order to meet the real situation. In the uncorrected situation, if we let the gradient of reflectivity near the critical angle in the s-polarization (at a large value of \( R_s \)) to be a constant, it is too large and too sensitive for the lower value of reflectivity. Therefore, the larger dithering in the grooves shown in Fig. 8(a) is due to the incorrect (larger) gradient. The profile dithering amplitudes shown in Fig. 8(b) in the grooves are smaller than in Fig. 8(a) and the profile is closer to the reference result (compared with the results of Dektak-6M), showing that the two gradients differ greatly. We are certain that the dithering shown in Fig. 8(a) jumped greatly in the grooves due to nonlinear errors.

(2) In the p-polarization

After nonlinear error compensation, the average height of the same grating of 20 lines/mm is 80.3 nm of the 2D surface profile in the p-polarization measurement as shown in Fig. 9(b). Comparing Fig. 9(b) with the uncorrected results shown in Fig. 9(a) the profile dithering amplitudes in the grooves are clearly reduced; however, the peak values of height have not visibly improved because the gradient of reflectivity near the critical angle in the p-polarization (at the larger value of \( R_p \), as in Fig. 6(b)) is too large and the region has very high angular sensitivity. However, the profile is closer to the reference result (compared with the results of Dektak-6M) than that in the s-polarization. Therefore, the nonlinear error compensation can improve the profile drawing since the average gradient of reflectivity greatly differs from that of the corrected gradient value.

3.2 Comparing the results of the proposed method with that of Dektak-6M

The commercial profiler Dektak-6M with a contact pin needle of 12.5 \( \mu \)m is used for the same grating measurement of 20 lines / mm and tested.

Fig. 10 shows the 2D measuring results, in which the average height is 82.8 nm. Table 2 presents the measurements of the same sample by the s- and p-polarizations. The measurement errors by using the s- and p-polarizations compared with the results from Dektak-6M are 4.1% and 3.3%, respectively. The error percentage for
each polarization is about 0.8%. The small angle sensitivity of the s-polarization can be used to measure a large scope of height (can be extended to more than 600 nm) while the high angle sensitivity of the p-polarization is suitable for measuring a small scope of height (less than 100 nm for the reason of experimental suggestion). We can see that the result of the three-dimensional (3D) profile of a 20 lines/mm grating in the s-polarization after the nonlinear error compensation (gradient correction) shown in Fig. 11(b) has a more even height distribution of grooves than the uncorrected result shown in Fig. 11(a).

Table 2 Various parameters in the s- and p-polarization

| Types of specimen | s-polarization | p-polarization |
|-------------------|----------------|----------------|
| Axial resolutions | 0.6 nm         | 0.5 nm         |
| Lateral resolutions | 1.54 μm       |                |
| Magnification     | 12.7           |                |
| Sensitivity (change/nm) | 0.00625      | 0.0078         |
| System errors     | 4.1%           | 3.3%           |
| Errors compared with | Dektak-6M      |                |

Fig. 11 3D surface profile pattern of a 20 lines/mm grating (a) before and (b) after nonlinear error compensation (gradient correction) in the s-polarization

3.3 Comparing the results of the proposed method with that of AFM

In another case, the grating sample is 40 lines/mm (Focal length of an objective of a confocal system of \( f_1 = 0.2 \text{ cm}; \) \( NA = 0.9; \) the second lens of \( f_2 = 10 \text{ cm}; \) \( M = -50; \) Measurement by the s-polarization). Figs. 13(a) and 13(b) show the 3D experiment results of the grating before and after gradient correction of this structure, the average heights of them are 75.6 and 76.9 nm, respectively. Large dithering amplitudes in grooves occur before gradient correction. The groove dithering clearly improves after gradient correction. This phenomenon is consistent with the measuring results of a grating of 20 lines/mm. Fig. 13(c), a 3D diagram of a grating measurement of 40 lines/mm by AFM, shows that this structure is closely approximate to Fig. 13(b) and the measured average height by AFM is about 79.4 nm. The error percentage of our best result compared with that of AFM is 3.2%.

Fig. 12 3D surface profile pattern of a 20 lines/mm grating (a) before and (b) after nonlinear error compensation (gradient correction) in the p-polarization

From Fig. 12(b), the 3D profile results of a 20 lines/mm grating in the p-polarization after the nonlinear error compensation, despite the even height distribution in grooves, uneven distribution in peak values appear. The 3D profile results are closer to the results of Dektak-6M than the uncorrected results shown in Fig. 12(a).

After nonlinear compensation of the gradient correction, the surface profiles of grooves and peaks should be closer to the actual profiles. A parallelogram prism may be considered an angular sensor to simplify the surface height equation into \( h = A \delta + h_0 \).
3.4 Applying the transformation for measurements of a transparent material and a biological sample

The sample in the third case is polystyrene (Focal length of an objective of a confocal imaging system of \( f_1 = 0.2 \text{ cm}; \ NA = 0.9; \) the second lens of \( f_2 = 10 \text{ cm}; \ M = 50; \ \theta = 7.6^\circ \); Measurement by the s-polarization). The gray level figure shots of TIR and critical angle images by CCD2 and CCD1 are shown in Figs. 14(a) and 14(b), respectively.

Perfectly overlapping these two images is very important for the calculation of reflectivity \( 2sR \). The distance between the sample and the objective is adjusted until the CCD’s image is clear prior to taking photos. The best adjustment method is rotating the parallelogram prism before reaching the critical angle. The black-and-white contrast ratio of polystyrene from the Fig. is clearly visible because of more sensitive reflectivity change near the critical angle.

Surface contour error is caused when the data of the test sample passes through surfaces of various optical components. Therefore, Fig. 14(c) shows the measured polystyrene 3D surface profile, including the system error for this structure; the system error is the height error induced by all the optical components and light source when the test sample is relieved from the system. Fig. 14(e) is the actual 3D surface profile after removing the system error shown in Fig. 14(d). Fig. 14(f) is the y-z view of polystyrene. The width is about 10 \( \mu \text{m} \) and the height is about 2.9 \( \mu \text{m} \).

For the sample in the fourth and final case is Euglena. The optical magnification is 120X. Fig. 15 is the actual 3D surface profile after removing system error. The maximum width is about 45 \( \mu \text{m} \) and the average height is about 794 nm. A suitable measured angle of incidence is 7.2°.

3.5 Height sensitivity:

Sensitivity \( (S) \) is defined as the partial differential equation below:

\[
S = \frac{\partial R_{s2}}{\partial h}
\]

Fig. 16 shows that the measuring scope of the angle of incidence is 5.7° ~ 8°. Therefore, we can obtain the best and the worst sensitivities. The best sensitivity is 0.012 (change/nm) when the...
external angle is 5.7°; the worst sensitivity is 0.000206 (change/nm) when the external angle is 8°.

Fig. 16 Sensitivity curve diagram corresponding to an external angle

3.6 Axial resolution (without considering the influence of the change of light intensity)

From Eq. (19), \( \Delta h_{\text{min}} \) refers to the axial resolution and \( \Delta R_{\text{S}}(\text{min}) \) refers to the minimum reflectivity change that can be judged by CCD. The CCD expression in gray scale value is 0~255 for 8 bits A/D converter; therefore, \( \Delta R_{\text{S}}(\text{min}) = 1/256 \). This minimum variation is also larger than the laser maximum intensity variation of 0.1%. Thus, the gravel level influence from a normal laser can be ignored. The formula for axial resolution \( \Delta h_{\text{min}} \) is as follows:

\[
\Delta h_{\text{min}} = \frac{\Delta R_{\text{S}}(\text{min})}{S}
\]  (19)

Fig. 17 shows the scope of axial resolution when the angle range is 5.7°~8°. Therefore, the best axial resolution \( \Delta h_{\text{min}} \) is, in theory, approximately 0.5 nm.

Fig. 17 Change curve of axial resolution that corresponds to an external angle

3.7 Lateral resolution:

The size of the CCD shown in this paper is 1024×768 (pixel × pixel) and the cell size is 4.65 \( \mu m \) × 4.65 \( \mu m \). The NA value of the objective used is 0.9. Lateral resolution is the resolving power and the formula is as follows:

\[
y_m = \frac{0.61\lambda}{NA}
\]  (20)

Therefore, the lateral resolution \( y_m \) toward the x-direction and y-direction is 0.42 \( \mu m \) for \( NA = 0.9 \) and \( \lambda = 0.6328 \ \mu m \).

4. Conclusion

This paper presents a simple, fast and real-time large-area measurement technique. AFM and commercial profilometer (Dektak-6M) take time-consuming measurements when compared with the proposed method. The proposed structure can be used for real-time large-scope measurements as long as the light intensity can be captured by CCDs. A parallelogram prism is used as an angular sensor to capture light intensity change with a combination of CCD image capturing technologies. We calculate the sample surface height change and map the 3D profile diagram by using the MATLAB program. The relationship between the measurable height range of the test sample and external angle is shown in Fig. 18.

Fig. 18 Change curve diagram of measuring height scope that corresponds to the external angle

Fig. 18 shows that the measurable angle of incidence range is 5.7°~8° for this structure’s measurement system. In this angle’s range, the maximum measurable height scope \( h = \pm 2.1 \ \mu m \) while the sample height shall be less than 4.2 \( \mu m \). Otherwise, the measurement error could be very large and cause measurement failure. The best sensitivity measurement angle is 5.6°~5.8°, but the measurable height scope is very small (less than 0.1 \( \mu m \)). The best and the worst axial resolutions are 0.5 \( \mu m \) and 19 \( \mu m \), respectively. This structure has such characteristics as high resolution, high sensitivity, simple structure, easy assembly, large-area measurement and fast measurement if the magnification is -50. This method can be used for nanometer level height measurements of samples and smaller cells in the future if the NA value or optical magnification is increased. This 3D optical microscope can be commercialized by combining it with automation features in the future.

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