FLUORESCENCE SIGNAL DETECTION FOR OPTICAL MICRO-THREE DIMENSIONAL MEASUREMENT BASED ON CONFOCAL MICROSCOPY

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Abstract:

We newly propose the optical micro-coordinate measurement technique based on the fluorescent signal. Surface position is detected based on fluorescent signal emitted from surface of structures by means of the confocal optical detection. Stimulated fluorophore emits fluorescence over wide angular ranges, allowing the confocal microscope to gather the fluorescent signal from steep surface (even from vertical sidewall). Thus this optical method is expected to measure three-dimensional shapes of smaller structures than 100 μm. Previously we reported the detection capability of a vertical surface based on the fluorescent signal with the confocal optical system. This paper deals with feasibility of three-dimensional measurement of micro-structures. Firstly, characteristic of detecting surface with various tilted angles were measured. As a substrate, the flat silicon wafer coated with the fluorescent dye thin film with a thickness of a few micrometers was used. Experimental result shows that the horizontal (0 deg.), vertical (90 deg.) and also undercut surface (120 deg.) could be successfully detected. At next, a cross-sectional shape of a metal wire was measured. A diameter of the wire was about 1 mm. The wire was also coated with fluorescent dye thin film. The result showed that the cross-sectional profile of the wire could be measured although the accuracy could not be ensured yet. We concluded that the proposed method manage to measure three-dimensional shapes of micro-structures.

Keywords: Dimensional measurement, optical probe, fluorescence, confocal microscopy

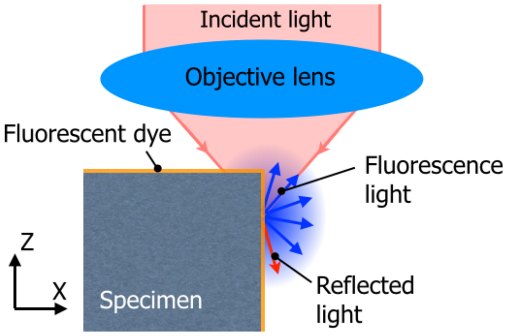
1. INTRODUCTION

Micro-dimensional metrology has been developed recently, for example, micro-coordinate measuring machine (micro-CMM) [1,2], scanning probe microscopy based dimensional metrology [3,4], micro-x ray coherence tomography (micro-XCT) [5] and optical techniques such as focus variation microscopy [6] and autofocus microscopy [7]. Micro-CMMs are one of the most reliable systems to assure dimensions of micro-parts [8]. However, the measurement speed is very slow and the probing system is the concern to develop the micro-CMM [9]. XCT is the promising technique but the measurement resolution is still as low as sub micrometers. Optical techniques are the most often used because of high measurement speed. Beside, some optical microscopies are capable of measuring the slopes as steep as 80 degrees if the surface is appropriately rough [6]. However, it is hard to measure dimensions of micro-parts such as hole diameter and groove width and edges.

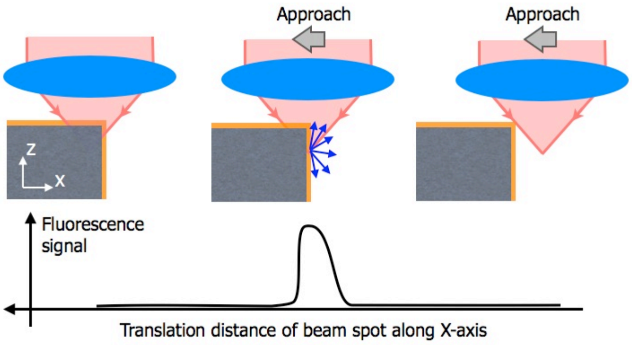
This study aims to develop the optical measurement technique to inspect three-dimensions of micro-parts on a basis of fluorescence confocal microscopy, so it is expected to measure as quickly as the conventional laser confocal microscopy. The measurement accuracy of several tens of nanometers is possible. In this paper, the basic principle of the proposed method is explained and also feasibility to three-dimensional measurement is experimentally investigated.

2. PRINCIPLE OF MEASUREMENT

Generally, optical microscopies are difficult to image the steeply inclined surface because the incident light is not reflected backward to the imaging system, which includes the information about surface, i.e. position, material, roughness. The proposed method utilizes a fluorescence signal to obtain the light. A fluorescent dye film is coated on the specimens and the fluorescent confocal microscopy is used to detect the surface. As seen in Fig.1, the laser is used to excite the fluorophore on the specimen, and then the



(a) Detection of surface based on fluorescence.



(a) Peaks in the confocal signal.
Fig. 1: Concept sketch of the measurement principle.

emitted fluorescence can be gathered by the fluorescent confocal optical system. This confocal signal is used to detect the surface of the specimen. The position of the surface can be determined by analyzing the peaks in the confocal signal. Fluorescence is emitted over wide angular range so that it is possible to measure steep slope such as sidewall [10].

3. EXPERIMENTAL SETUP

3.1 Experimental setup

Experiments were conducted to confirm that surface sensing could be achieved by using fluorescence signals. Fig. 2 shows the schematic of the fluorescent confocal optical system. An argon-ion laser (λ = 457 nm) was used to excite fluorescent dye. The laser beam was collimated and adjusted to enter the objective lens (NA 0.80, WD 2 mm). The specimen was put on the piezo-stage, i.e., the focal spot was fixed and the specimen was displaced horizontally and vertically. The stroke of the piezo-stage was ± 8 μm. The fluorescence signals were gathered by the objective lens, and the dichroic mirror and long-pass filter allowed it to reach the pinhole. The photomultiplier tube (PMT) was used to detect the fluorescence. The CCD was used to observe the beam spot on the specimen in order to adjust the optical arrangement.

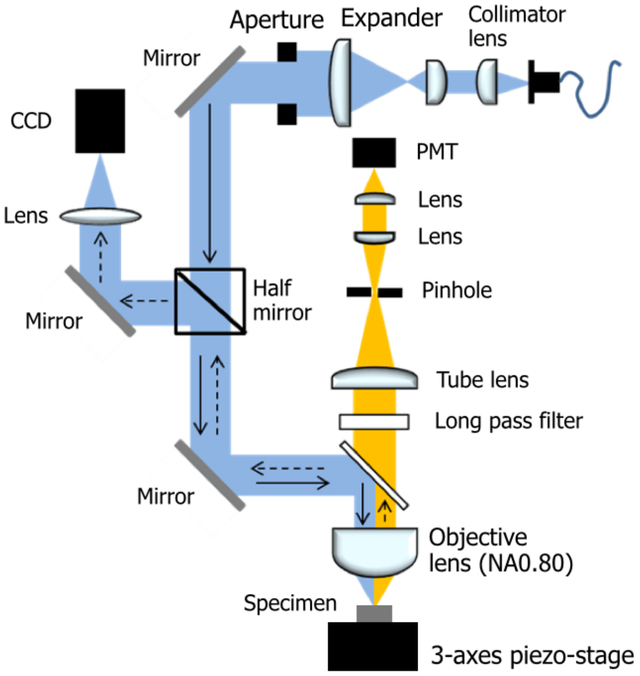


Fig. 2: Experimental setup.

3.2 Preparation of fluorescent dye film

Limited materials have fluorescent properties and the exciting wavelengths of them are different. Therefore, we opted to coat the fluorescent dye film on the specimens to unify the fluorescence properties. Cleaning by the ultrasonic bath can easily remove the fluorescent dye film. First, the fluorescent dye (Fluolid-W-Orange, fluorescent wavelength: 610 nm, International Science & Technology Co., Ltd) [11] was dissolved in a chloroform solution with concentration of 10 mM. Then, a drop of the solution (50 μl) was put on a silicon substrate, and the thin fluorescent dye film was produced by means of spin coating method. The thickness of the produced film was around 50 nm (the rotation speed 500 rpm). A smooth silicon wafer (surface roughness less than 1 nm in Ra) was chosen as the flat surface substrate to avoid the scattering of light on the surfaces (Chapter 4). A metal wire was used as the specimen for three-dimensional measurement (Chapter 5). To coat the metal wire, a drop of the solution on the wire was dried for about 5 minutes without the spin coating. In this case, the thickness of the dye film was less than 1 μm.

4. FLUORESCENCE DETECTION

4.1 Flat surface detection

Flat surfaces with different inclined angles were firstly detected. The angle was varied from 0° to 120°. 0° means the surface perpendicular to the optical axis. 90° means vertical surface (sidewall), which also indicates that more than 90° means undercut surfaces.

The silicon wafer was used as the specimen, which was fixed by handmade fixtures as shown in Fig.3. The protractor was employed to determine the surface angled with accuracy within ±0.5°. The piezo-stage was used to scan the surface. Specimen was scanned along with optical axis for the surface inclined 0° to 40° and scanned perpendicular to the optical axis for the surface inclined 50° to 120°. The scanning speed was set to 32 μm/s and the sampling rate of the fluorescent signal was 100 Hz.

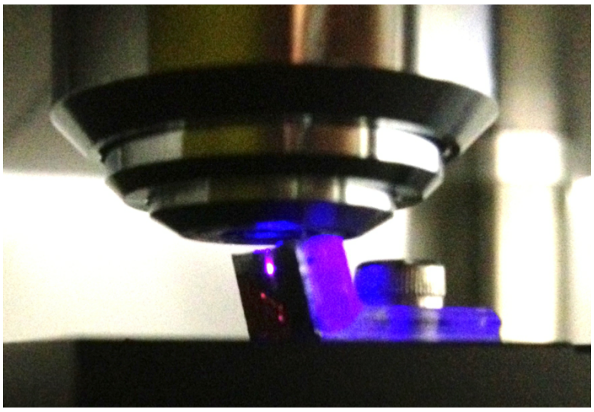
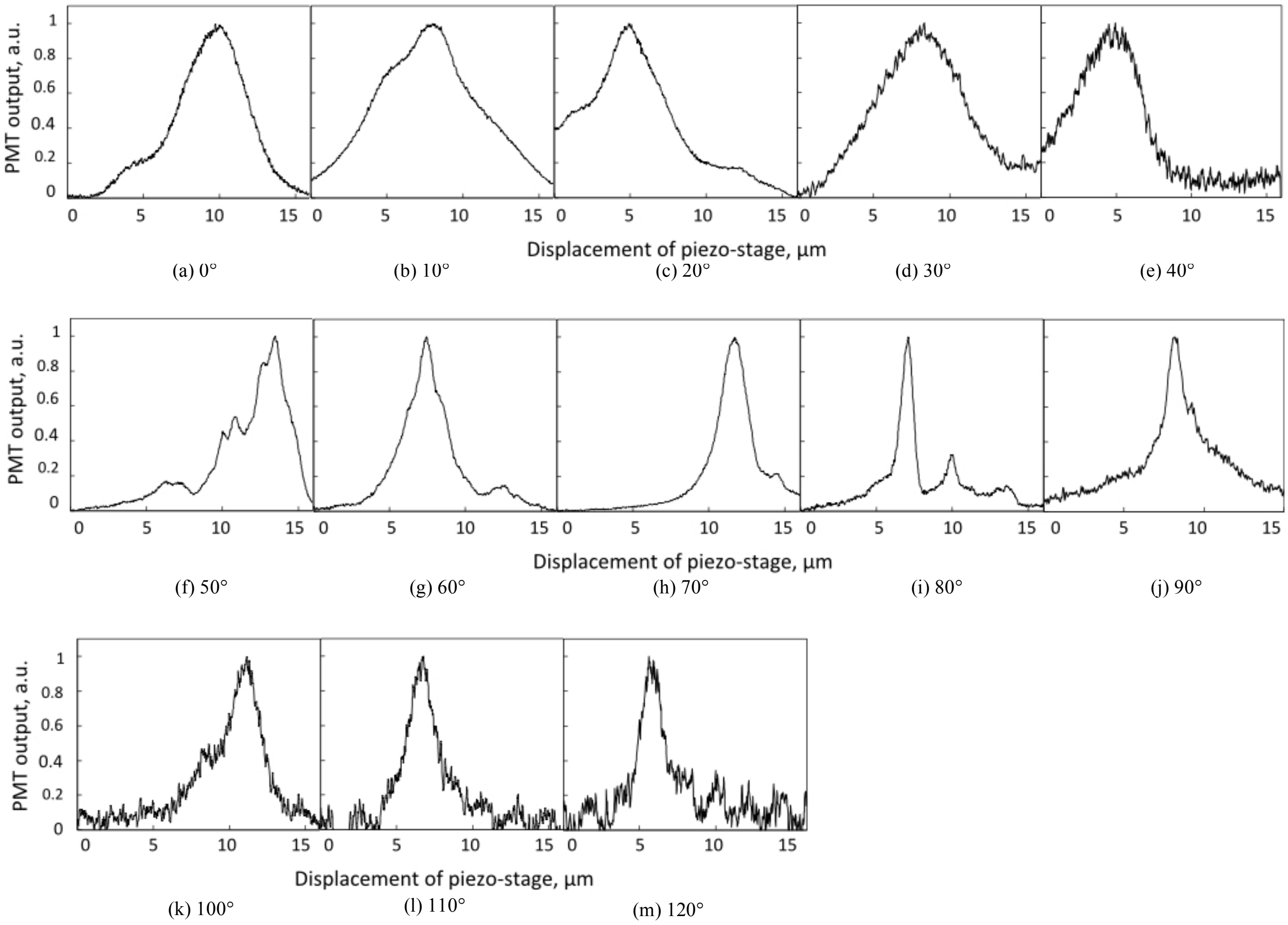


Fig. 3: Photograph of measurement (110° inclined surface).

The results are shown in Fig.4. These proves that the all surface inclined 0° to 120° could be detected with measuring the fluorescence. It is noteworthy that even undercut surfaces, which are surface with tilted angle of 100° to 120°, could be detected, although the signal to noise ratio is lower than others.

Full width at half maximum (FWHM) of the peak of confocal signals is evaluated. FWHM for the surface inclined 0° to 40° is around 5 μm. The peaks are getting narrower with increasing the tile angle until 90°, which is



(a) 0° (b) 10° (c) 20° (d) 30° (e) 40°

(f) 50° (g) 60° (h) 70° (i) 80° (j) 90°

(k) 100° (l) 110° (m) 120°

Fig. 4: Fluorescent confocal signal for surface inclined different angles.

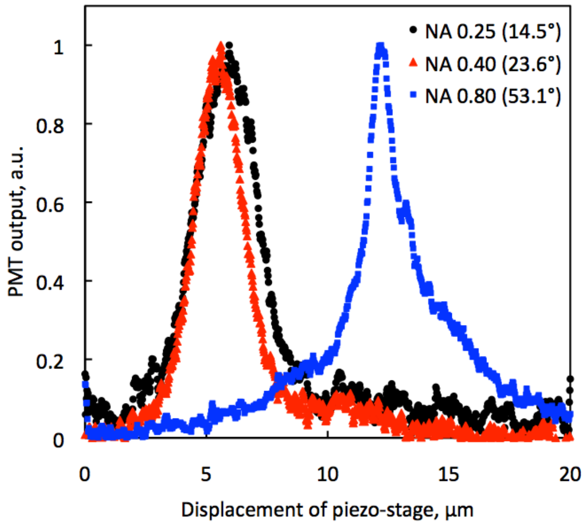


Fig. 5: Fluorescent confocal signal with the objective lens of different numerical aperture.

possibly because the part of beam is shaded by the specimen when the specimen is approaching to the beam waist of focused beam. During detection, interactive conditions such as incident laser power, intensity distribution of focused beam and detectable angular range of emitted fluorescence by the objective lens are changing. And also the conditions depend on the specimen's shapes and numerical aperture (NA) of the objective lens. These behaviors of confocal signals should be investigated more to analyze the peak as future work.

4.2 Influence of collecting angle

For vertical surface, which is surface inclined 90°, surface detection was implemented to compare the different shading conditions. Incident angle of the beam was changed with the NA of the objective lens. Larger NA of the objective lens

Table 1: Specifications of the objective lens and the result of fluorescent signal detection.

Specifications of objective lens and incident beam			
NA	0.25	0.40	0.80
Working distance	6.8 mm	10.4 mm	2.0 mm
Max. incident angle	14.5°	23.6°	53.1°
Beam waist	2.79 µm	1.40 µm	0.70 µm
Result of surface detection			
FWHM	3.07 µm	2.30 µm	2.05 µm

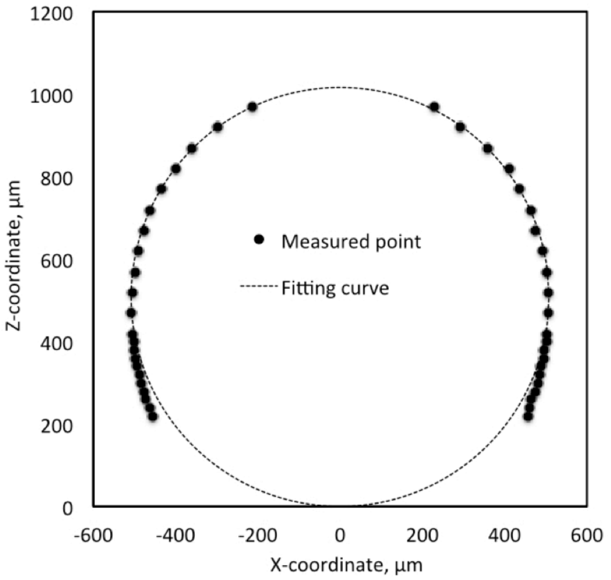


Fig. 6: Three dimensional measurement of metal cylinder.

can collect the larger amount of the fluorescence, but is shaded larger area. Specifications and related parameters are listed in Table 1.

Surface detections were carried out at 1 mm depth from the surface edge of the specimen. The experimental results are shown in Fig.5 and FWHMs are summarized in Table 1. It was possible to detect surface with the objective lens with low NA (0.25), whose confocal signals are similar to the one of NA 0.40 whereas the confocal signal for the objective lens of NA0.80 is different. From this result, surface detection is more sensitive at higher NA of the objective lens.

5. DIMENSIONAL MEASUREMENT

A metal wire was measured to examine the properties of three-dimensional measurement. A nominal diameter of the wire was 1 mm. Thickness of the coated fluorescent dye film was less than 1 µm. The wire was displaced horizontally along X axis for measurement by the fine stage at different positions in Z-axis. Micrometers are used to measure the coordinate value of surface detection. NA of the objective lens was 0.80 in this experiment.

Fig.6 shows the measured result. Black plot indicates the measured points and dot line is the regression circle based on the measured point. A diameter of the regression circle was 1.018 mm. It seems that upper side of the wire could be measured properly but the lower side was not. At lower side, it was able to detect the fluorescence but the detected positions were not reflected the surface position of the wire.

6. CONCLUSION

We investigated optical technique to measure 3-dimensional shape of the miniature structures based on the fluorescence confocal microscope.

Surface detections were successfully possible for surface inclined 0 to 120°. In order to know the surface position accurately, more study is necessary to analyze the peak of the confocal signal, influential factors of which are NA of the objective lens, surface shape and so on.

Dimensional measurement reveals that the surface could be measured from 0° to 90° (horizontally to vertically). For more than 90°, however, detected position of the surface was obviously wrong, which must be corrected.

This measurement technique is expected to use for measuring a cutting tool immersed with cutting oils and an electrode of an electro discharge machine.

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REFERENCES

[1] R.K. Leach, “Fundamental principles of engineering nanometrology,” Elsevier, Oxford, 2009.

[2] A. Weckenmann, T. Estler, G. Peggs and D. McMurtry, “Probing systems in dimensional metrology”, *CIRP Ann-Manuf. Techn.*, vol.53, issue 2, 657–684, 2004.

[3] G. Dai, W. Haßler-Grohne, D. Huser, H. Wolff, H.-U. Danzebrink, L. Koenders, H. Bosse, “Development of a 3D-AFM for true 3D measurements of nanostructures, *Meas. Sci. Technol.*, vol.22, 094009, 2011

[4] J. A. Kramar, R. Dixson, N.G. Orji, “Scanning probe microscope dimensional metrology at NIST”, *Meas. Sci. Technol.*, vol.22, 024001, 2011

[5] J.P. Kruth, M. Bartscher, S. Carmignato, R. Schmitt, L.De Chiffre and A. Wechkenmann, “Computed tomography for dimensional metrology”, *CIRP Ann-Manuf. Techn.*, vol.60, issue 2, 821–842, 2011.

[6] R. Danzl, F. Helmli, S. Schereret, “Focus variation –A new technology for high resolution optical 3D surface metrology”, Proc. 10th International conference of the slovenian society for non-destructive testing, 484, 2009

[7] R.K. Leach, “Optical measurement of surface topography”, Springer, Berlin, 2011.

[8] A. Küng, F. Meli, R. Thalmann, “Ultraprecision micro-CMM using a low force 3D touch probe”, *Meas. Sci. Technol.*, vol.18, 319, 2007

[9] A. Weckenmann, P. Peggs, J. Hoffmann, “Probing systems for dimensional micro- and nano-metrology”, *Meas. Sci. Technol.*, vol.17, 504, 2006

[10] M. Michihata, A. Fukui, T. Hayashi, and Y. Takaya, “Sensing a vertical surface by measuring a fluorescence signal using a confocal optical system”, *Meas. Sci. Technol.*, vol. 25, 064004, 2014.

[11] T. Kanemaru, K. Hirata, S. Takasu, S. Isobe, K. Mizuki, S. Mataka and k. Nakamura, “A fluorescence scanning electron microscope”, *Ultramicroscopy*, vol.109, 344–349, 2009