HEIGHT MEASUREMENT OF A PARTICLE IN EVANESCENT FIELD CONTROLLING PENETRATION DEPTH

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Abstract:

The total internal reflection (TIR) microscopy is one of the most widely used microscopy techniques in the fields such as bioscience, surface science, and micro-devices engineering (namely, MEMS, μ-TAS, etc.), because of the advantage of incomparably high contrast image formation characteristics near the TIR surface. The evanescent light is a localized form of electromagnetic field near the TIR surface and, because of this unique feature, the optical analysis or measuring method using it is considered a potentially effective tool in the surface observation.

It should be noted, however, that the use of this form of light in the microscopic measurement has traditionally been limited only to the "two-dimensional" imaging, and that the possibility of measurement along the "vertical" direction using it has attracted little attention.

The aim of our research is to enable the 3D image-reconstruction of samples in the evanescent field. As the first step, we propose a completely new particle height measurement method in the evanescent field. This method is based on the fact that the power of scattered light intensity from a sub-micrometer (possibly, fluorescent) particle in the evanescent field strongly depends both on its distance from the TIR surface and also on the penetration depth of the field. By changing the penetration depth and detecting the scattered light simultaneously, the particle's height from the TIR surface could be calculated.

As a verification of the proposed method, an FDTD simulation and a preliminary experiment were carried out. Results support the validity of the proposed method.

Keywords: Evanescent field, Height measurement, Penetration depth, TIRF microscopy, Intelligent measurement

1. INTRODUTION

The total internal reflection (TIR) microscopy is widely used in the fields such as micro-engineering, surface science and bioscience, as one of the strongest tools for a high-contrast observation near the glass surface.

Evanescent light is a form of localized photon, which is formed when the light is total-internally reflected at the border of two mediums with different refractive indices. The electromagnetic energy at the TIR surface is localized in the area of around several hundred nanometers from the TIR surface, i.e, about the scale of the wavelength of the incident light. Using such electromagnetic filed as an illumination, high selectivity of observation area near the TIR surface, high S/N ratio and image contrast are achievable with the TIR microscopy [1].

Traditionally, the use of this form of light in the microscopic measurement has been limited mainly to the two- dimensional imaging of samples near the TIR surface. While many 3D optical imaging or profiling methods are proposed for the "transmitted light" microscopy, few are presented for that using the evanescent light. If such a method were established, it would become an effective tool in the fields mentioned above.

In respect of lateral resolution improvement, we have been working on the development of image retrieval method based on structured illumination phase shift [2].

In similar fashion, to our height measurement method we aim to apply the concept of super-resolution strategy, in which the resolution of the optical image is improved through a combination of illumination-modulation technique and successive computational processing of multiple images. In this method, the illumination modulation is realized by the control of the penetration depth of the evanescent field. Because the scattered intensity of light from an object depends on the intensity of electromagnetic field around it, by controlling the penetration depth of the evanescent field and simultaneously detecting the power of the scattered light, we can obtain the height-information of samples near the TIR surface. Detailed explanation of the method is given in chapter 2.

In order to verify this method, targeting on the sub-micrometer particles as sample objects, we carried out an FDTD simulation and a preliminary experiment. Through these verifications the validity of the proposed method is confirmed.

2. PRINCIPLE OF THE PARTICLE HEIGHT MEASUREMENT METHOD BASED ON PENETRATION DEPTH CONTROL

As we mentioned above, the principle of the height measurement method is based on the fact that the intensity

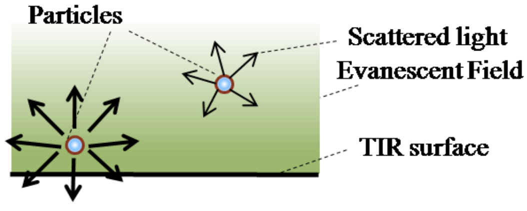

Fig.1: Scattered intensity of light from a particle in the evanescent field depends on its height from the TIR surface

of the evanescent field decreases exponentially with z, the distance from the TIR surface (Fig. 1). This means that the intensity of the scattered light from a particle in this region strongly depends on the height from the TIR surface.

General concept of the method is shown in Fig. 2. If the size of the particle is much smaller than the penetration depth of the evanescent field d(θ) (θ; the incident angle of light), then the total power of scattered light from the particle also can be written in a form: exp(-z/d(θ)). When this is observed through an optical system, the intensity is multiplied by a factor of psf(z) (point spread function). The observed intensity, therefore, is given by;

$$S(\theta, z) = S(\theta, 0) \cdot \exp\left(\frac{-z}{d(\theta)}\right) \cdot H(z) \cdot psf(z). \quad (1)$$

Here, H(z) is a term representing the effect of interference between the particle and the surface. Equation (1) suggests that the scattering power depends not only on the height of the particle but also on the penetration depth of the evanescent field, d(θ): This means that by regarding d(θ) as a parameter and varying it, we can modify the scattered intensity from the particle (Fig. 2). Note that d(θ) is a function of the incident angle. Scattering powers observed for $\theta = \theta_1$ and $\theta = \theta_2$ ($\theta_1 = \theta_2$), therefore, are of different values and are given by the following equations;

$$S(\theta_1, z) = S(\theta_1, 0) \cdot \exp\left(\frac{-z}{d(\theta_1)}\right) \cdot H(z) \cdot psf(z). \quad (2)$$

and

$$S(\theta_2, z) = S(\theta_2, 0) \cdot \exp\left(\frac{-z}{d(\theta_2)}\right) \cdot H(z) \cdot psf(z). \quad (3)$$

Here we assume that both H(z) and psf(z) are independent of the incident angle θ, that they only relate to the particle's height and the equipment's image formation characteristics. Under this assumption, by dividing Eq. (2) with Eq. (3), we can eliminate these terms. Rearranging it for z, we obtain the following equation.

$$z_{cal} = \frac{d(\theta_2)}{\left(1 - \frac{d(\theta_2)}{d(\theta_1)}\right)} \ln\left[\frac{S(\theta_1, z)}{S(\theta_2, z)} \frac{S(\theta_2, 0)}{S(\theta_1, 0)}\right]. \quad (4)$$

Terms S(θ_1, z) and S(θ_2, z) are the observed intensities, while terms d(θ_1), d(θ_2), S(θ_1, 0) and S(θ_2, 0) are the calculated values for given θ_1, θ_2, and the two refractive indices of the mediums, n_1 and n_2.

Equation (1) contains functions of z, H(z) and psf(z),

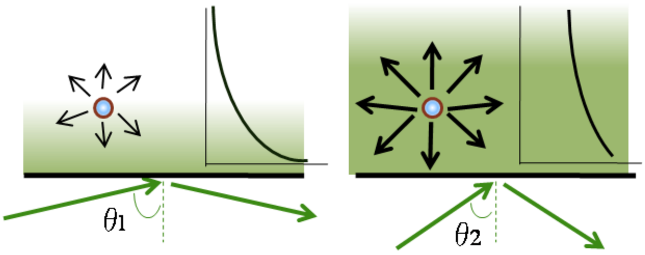

Fig. 2: The scattered intensity from a particle in the evanescent field at $\theta = \theta_1$ (left) and θ_2 (right).

which in general are unknown. Unless their forms are precisely understood, we cannot calculate the height only from the scattered intensity measured for a single incident angle. However, by measuring intensity for two (or more) incident angles, we could calculate the particle height without any previous knowledge for H(z) and psf(z), which is the greatest advantage of this method.

3. VERIFICATION OF THE PROPOSED METHOD BY FDTD SIMULATION

In order to verify the proposed method, an FDTD (finite-difference time-domain) simulation [3] was carried out. Here, we aim to see the inclination of the proposed method using typical values for the parameters. Simulation setup and parameters are shown in Fig. 3. A particle (diameter: 50nm) is placed in the lower refractive index medium (n_2=1.0), distanced from the surface by z_0 (0 to 250nm, 50nm step), and illuminated by the evanescent light. From the side of the higher refractive index (n_1=1.6) medium, the light (wavelength: 532nm) is incident to the interface of two mediums, making the incident angle θ (=45deg, 60deg), which is chosen to be larger than the critical angle θ_c (=38.8deg), so that the TIR condition is satisfied. A typical image during the simulation representing the time-average of the scattered electromagnetic field around the particle (height=200nm) is shown in Fig. 4.

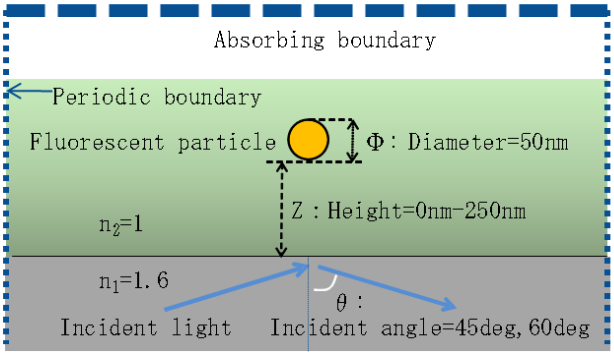

Fig.3: Schematic diagram of simulation setup

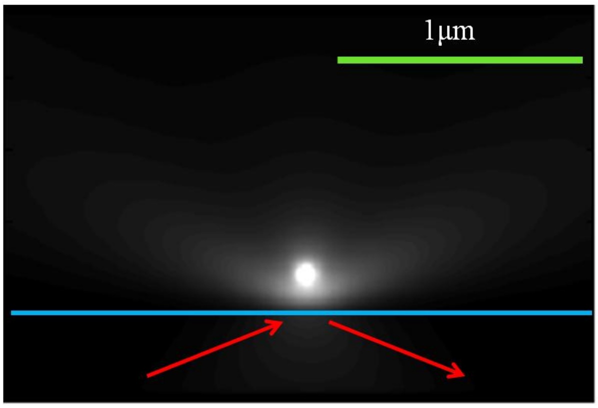

Fig.4: A typical image representing the electromagnetic field around the particle

Table 1: Scattered intensity obtained in the simulation [a.u]

Setup height[nm]	0	50	100	150	200	250
θ =45[deg]	100	83.3	58	30.3	13.4	6.51
θ =60[deg]	38.7	19.8	8.51	2.71	0.71	0.2

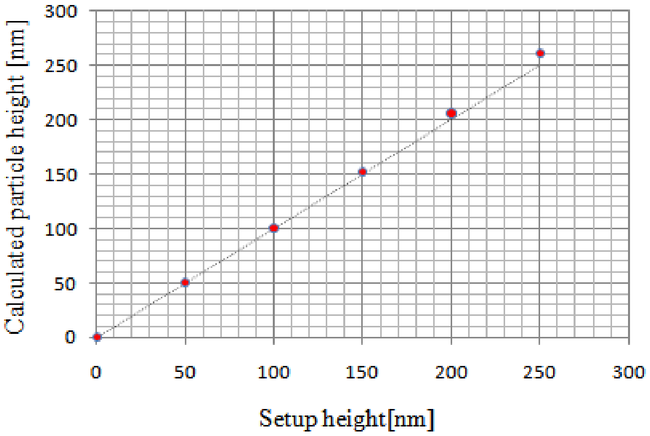

Fig.5: Particle height calculated from the simulation data

The scattered intensity obtained in this simulation is shown in Table 1. Here we defined the time average of the electromagnetic energy absorbed in the upper boundary to be the observed scattered intensity. We can see from the data that the intensity decreases with the arranged height of particles and, more importantly, that the variation of the intensity change clearly differs from each incident angles. The height calculated with this data is shown in Fig. 5. The horizontal axis represents arranged height of the particle z_0 and the vertical one calculated height, z_{cal}.

The result shows a tendency that the higher z_0 is set, the less accurate z_{cal} becomes. Seemingly, though, they well agree with all the set heights with the accuracy of around 10 nanometers. This result suggests that the proposed method successfully gives the particle height within the region of 1/2 of wavelength, with the overall accuracy below 10%. Further qualitative examination will be performed in future works about the causes for this error.

4. VERIFICATION WITH FUNDAMENTAL EXPERIMENT

A fundamental experiment was also carried out as the verification of the proposed method. As the preliminary trial, we prepared a fundamental apparatus and a sample containing the fluorescent reagent microspheres, and aimed to see if the desired scattered intensity change actually shows up in the data taken with this penetration depth control method. Also, using the data obtained in the experiment, particle height was calculated with the proposed method and was compared with the actual height profile measured by AFM.

4.1 Verification experiments – equipment setup

The schematic diagram of the apparatus is shown in Fig. 6. A prism of high refractive index (*n*=1.78) is used to produce the evanescent light. On the surface of the prism is applied the immersion oil, then on it the sample coverglass. The refractive indices of the prism, the immersion oil, and the coverglass are the same (*n*=1.78). The incident laser beam (wavelength=488nm) comes out of an optical fiber, which is attached on the rotation stage so that the incident angle could be controlled. The polarization of the incident light is chosen to be s-polarization. The scattered light is condensed with an objective lens (Olympus, NA=0.90, f=3mm), then forms an image (250 times magnified) on the cooled CCD camera (Bitran, BU-41L) through a tubelens.

The observed images are sent to a PC through USB interface. After processing the data (averaging and noise substitution), the height of the observed particle was calculated using the proposed method. A typical image of intensity change with regard to the incident angle observed in this setup is shown in Fig. 7.

4.2 Verification experiments – sample preparation

In the verification experiment, the actual height profile of particles must be known in advance with high reliability, in order to compare with the calculated height after the measurement. For this, we aimed in creating such particle distributions with a special type of rapid prototyping method using the evanescent light [4]. Here, a photopolymer resin (*n*=1.52) was cured with the evanescent light, so that the solid resin would form a layer of as thin as several hundred nanometers (fig.8). By measuring the height of this layer with an AFM in advance and later scattering the fluorescent particles (diameter=200nm, emission wavelength=612nm)

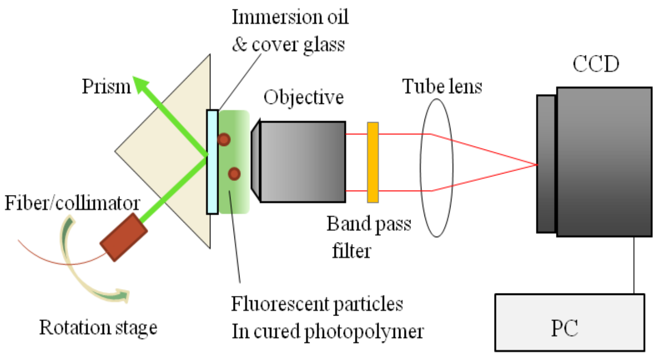

Fig.6: Schematic diagram of the experiment apparatus

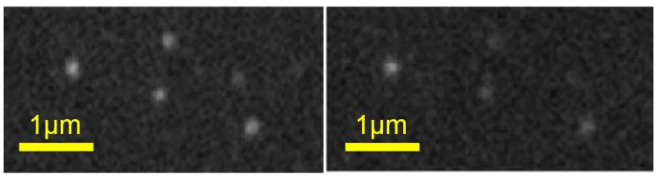

Fig. 7: A typical image showing the scattered intensity change with regard to the incident angle θ:
θ = 57.9 degrees (left), and
θ = 59.2 degrees (right).

upon it, we obtained the actual height profile of the particles. In addition, after profiling with AFM, whole resin area was enclosed with the same photopolymer and cured again, this time with the transmitted light. By surrounding the fluorescent particle with same refractive index medium, i.e, photopolymer in this case, the formation of the evanescent field with a "correct" form (exp(-z/d(θ))) near the particles is guaranteed.

This way, a cured resin area of around 100μm×100μm in lateral dimension and several tens of micrometers in height, containing in its lowermost region the height-known fluorescent particles is prepared.

4.3 Experiment Results

Sample thus prepared was observed in the experimental apparatus explained above. The result for three particles at different heights is shown in Fig. 9. The horizontal axis represents the height of each particle measured with AFM, and the vertical one calculated height. Accuracy of the data is around 10%. Here, the data was taken with the set of two incident angles, θ_1 = 57.9deg and θ_2 = 59.2deg. It is predicted that the combination of two incident angles used in the measurement may have an effect on the accuracy of the calculated height. In future works, the reliability of the system will be improved for a more qualitative verification of the proposed method.

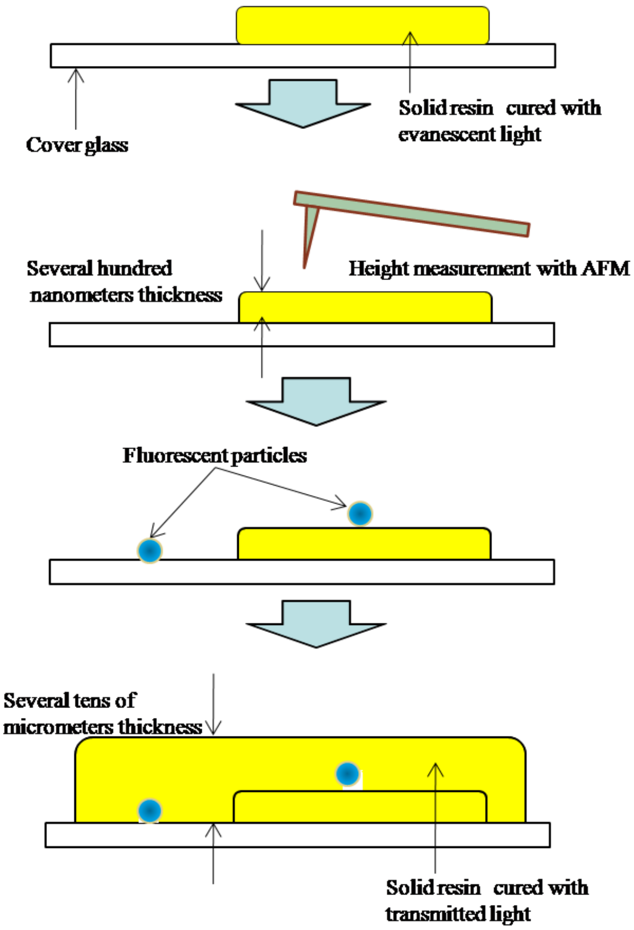

Fig.8: Procedure of the sample setup

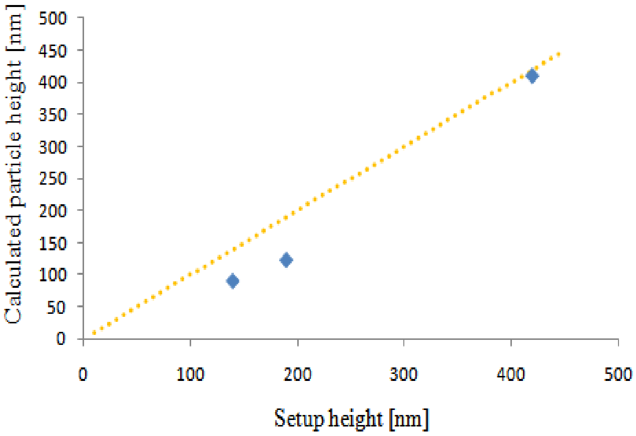

Fig.9: Particle height calculated from the experiment data

5. CONCLUSION

A novel height measurement method for a particle in the evanescent field, based on the control of the penetration depth was proposed. As verification, we carried out an FDTD simulation and a fundamental experiment. The results support the validity of the proposed method. We will be performing an elaborate experiment in future works for a more qualitative verification of the method.

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