Total internal reflection is an optical imaging technique for nanoparticle tracking and observation employing the scattered light from an evanescent field near the interface or reference surface. Generally, the nanoparticle behavior is the three-dimensional Brownian motion in an aqueous medium. The motion can be traced by an optical microscopy, but it cannot be traced by an electron microscopy technique. In the three-dimensional nanoparticle moving position, the $X$ and $Y$ positions are parallel to the surface, which can be traced by the general microscopy techniques. However, the height position $Z$ of a nanoparticle perpendicular to the surface could not be traced without the longitudinal scanning method. Here, a novel method is proposed to investigate the 3D position of nanoparticles by applying multi-wavelength evanescent fields microscopy, which has a high spatial resolution in the $Z$-direction without longitudinal scanning. This paper focuses on the verification of measurement in the $Z$-direction. A piezoelectric actuator was employed to control the nanoparticle displacement in height $Z$. Standard polystyrene 100 nm particles were randomly adhered on a spherical tip that connected with the piezoelectric actuator. The spherical tip was essentially made from an optical adhesive ($n = 1.348$) with a refractive index close to the water for decreasing the unnecessary signal from the tip-self during nanoparticle observation in the water. The proposed method could obtain the multi-wavelength scattering lights from the observed nanoparticles by an 8-bit color camera with higher than 50 frames per second recording to investigate the 3D nanoscale tracking. The $X$ and $Y$ positions of nanoparticles were determined by the centroid of the scattering light intensities. The height $Z$ was determined from the logarithm ratios between the detected scattering light intensities of both wavelengths. The measurement repeatability of the absolute difference in height between nanoparticles could be measured less than ±16 nm by using the proposed method. The penetration height measurement ability range was approximated at 250 nm from the reference surface.

**Keywords:** 3D nanoscale tracking, longitudinal position, polystyrene nanoparticle, optical multi-wavelength, evanescent field microscopy

1. **Introduction**

Nanoparticles smaller than 100 nm have been developed and used in the chemical mechanical polishing (CMP) process to planarize a substrate surface in the semiconductor manufacturing wet process. The nanoparticle behavior is three-dimensional (3D) at high-speed movement in a liquid solution during the CMP process. Generally, the polishing phenomenon of a nano-abrasive particle occurs only near the surface substrate of a wafer. The polishing phenomena were evaluated by comparing the nanoparticle size and shape before and after the polishing process [1]. This was due to the inability to observe the phenomena occurring through the polishing process, which inspired us to develop a real-time observation apparatus on an optical microscopy system. Optical microscopy imaging of individual nanoparticles can reveal their real-time behavior during the process. The ISO standard calibration method for light scattering is based on single-particle measurements, which measure the particle number and size distribution in a liquid. The typical size range of particles measured by this ISO standard method is between 0.1 μm and 10 μm [2]. Furthermore, several studies have applied evanescent field microscopy to detect the scattering light from nanoparticles near a surface with high image contrast [3–9]. Our previous study determined the sub-100 nm particle size without fluorescent labeling by applying evanescent field microscopy to our developed optical apparatus [10].

To date, state-of-the-art nanoparticle tracking analysis (NTA) methods have two-dimensional (2D) tracking ($X$ and $Y$) methods in a longitudinal direction without longitudinal scanning. Here, a novel method could obtain the multi-wavelength scattering lights from the observed nanoparticles by an 8-bit color camera with higher than 50 frames per second recording to investigate the 3D nanoscale tracking. The $X$ and $Y$ positions of nanoparticles were determined by the centroid of the scattering light intensities. The height $Z$ was determined from the logarithm ratios between the detected scattering light intensities of both wavelengths. The measurement repeatability of the absolute difference in height between nanoparticles could be measured less than ±16 nm by using the proposed method. The penetration height measurement ability range was approximated at 250 nm from the reference surface.

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and $Y$) high resolution, but the third longitudinal dimension ($Z$) has a low resolution owing to the depth of field (DOF) limitation of optical microscopy [11]. In this instance, the nanoparticle $X$ and $Y$ coordinates are parallel to the substrate surface. The distance from the substrate surface to the nanoparticle is the height $Z$ in the third dimension.

To achieve higher resolution in the third dimension, we have proposed a novel method for 3D nanoscale tracking of individual particles smaller than 100 nm by applying multi-wavelength evanescent fields to our developed apparatus [12–14] because the single-wavelength evanescent field was not accurate enough to determine the height $Z$. However, our experiments evaluated the 3D tracking of Brownian motion in controlling the height $Z$ of nanoparticles without image scanning. Moreover, an optical scanning method was used to measure the height of a single nanoparticle near the surface by controlling the evanescent field penetration depth using the finite-distance time-domain (FDTD) method [15]. A piezoelectric actuator converts an electrical signal into a precisely controlled physical displacement that can be employed to calibrate the height $Z$ of fluorescent nanoparticles by applying the total internal reflection fluorescence microscopy (TIRFM) technique [16]. However, our challenge is to measure the 3D position of moving nanoparticles without fluorescent labeling for utilization in several fields of science and industry in the near future.

Therefore, this study is focused on the height $Z$ verification of an individual nanoparticle to evaluate the effectiveness of the proposed method. Multi-wavelength evanescent field microscopy has been proposed to measure the 3D position of nanoparticles in water. Standard polystyrene (PS) 100 nm particles were used because of their highly uniform particle size and high dispersity. First, the nanoparticles adhered to the spherical tip. The spherical tip was made from an elastic material, which has a refractive index ($n$) close to the water to decrease the signal noise while observing the nanoparticles. As a result, the spherical tip was invisible when dipped into the water. Then, the adhered nanoparticles on the spherical tip were connected to the piezoelectric actuator to control the displacement of the nanoparticles at height $Z$ in water. However, the absolute height $Z$ from the reference surface was unknown. Thus, we compensated the height $Z$ for analyzing the displacement and absolute difference height of the nanoparticles. Consequently, the proposed method could verify the measurement repeatability of an absolute difference height, $\Delta Z$, between nanoparticles.

2. Near-Surface Observation of Nanoparticle

The evanescent wave is generated from the total internal reflection (TIR) when light is internally reflected off an interface. This is caused by illuminating the light from the higher refractive index $n_1$ material to the material with a lower index $n_2$ at an incident angle $i$ greater than the critical angle $\theta_c$, as shown in Eq. (1). Snell’s law is used to describe the relationship between the incidence and refraction angles, as shown in Eq. (2). However, the evanescent field is created at the interface of the lower refractive index $n_2$, a side that does not propagate in free space. It exists near the surface within a few hundred nanometers, as shown in Fig. 1.

$$\theta_c = \sin^{-1}\left(\frac{n_2}{n_1}\right) \quad \ldots \ldots \ldots (1)$$

$$n_1 \sin i = n_2 \sin \theta_c \quad \ldots \ldots \ldots \ldots (2)$$

2.1. Single-Wavelength Evanescent Field

Generally, the evanescent light intensity decays exponentially with distance from the reference surface [10]. This is observed when the particle of size $D$ moves into the evanescent field near the reference surface. The scattering light intensity from nanoparticles decreases when the nanoparticle height is increased, as shown in Fig. 2, where $Z$ is the distance from the reference surface to the bottom of the particle, $\lambda$ and $I_0$ are the laser wavelength and laser intensity, respectively. $\xi$ is a parameter that relates to the laser wavelength and Snell’s law, which corresponds to the inverse variation of the evanescent wave penetration height $Z_p = 1/\xi$, in which the $\xi$ can be determined using Eq. (3).

$$\xi = \left(\frac{4\pi}{\lambda}\right) \sqrt{n_1^2 \sin^2 i - n_2^2} \quad \ldots \ldots \ldots \ldots (3)$$

The amount of light scattered from a particle in an evanescent field can be obtained and explained by a...
A Novel Method for 3D Nanoscale Tracking of 100 nm Polystyrene Particles in Multi-Wavelength Evanescent Fields

2.2. Multi-Wavelength Evanescent Fields

It can be seen from the preceding discussion that the single-wavelength evanescent field could not accurately determine the height $Z$. Therefore, we have added one more laser wavelength to generate multi-wavelength evanescent fields to localize the height $Z$ of nanoparticles at a higher resolution [12–14]. Both evanescent fields are emitted perpendicular to the reference surface at different penetration heights ($Z_{p1} > Z_{p2}$). Optical microscopy systems can accumulate and detect scattered light from nanoparticles in evanescent fields. The scattering light intensities of both wavelengths from the same size particles are different when the height $Z$ of the particles is changed, as shown in Fig. 4. The scattering light intensities of both wavelengths are exponentially increased when the height $Z$ is decreased. Hence, the scattering light intensities for both wavelengths ($I_{λ1}, I_{λ2}$) are expressed in Eqs. (7) and (8). Further, the height $Z$ of the unknown particle size $D$ can be obtained by substituting the ratio of the scattering light intensities ($I_{λ1}/I_{λ2}$) into Eq. (9), where $k$ is a constant compensating for the height $Z$ from the experiments. The laser intensities $I_{01}, I_{02}$ are irradiated at incident angles $i_{1}, i_{2}$ [deg], in which both laser beams are total internally reflected from the reference surface, and $ξ_1, ξ_2$ are composed of total internal reflection parameters. Thus, the height $Z$ in the third dimension can be determined by a two-dimensional image of the scattered light on an optical multi-wavelength microscopy system.

\[
I_{λ1}(D, Z) \propto I_{01} \left( 1 - \sqrt{1 - NA^2} \right) e^{-ξ_1Z} \frac{D^2}{2} e^{-ξ_1Z} \quad \ldots \quad (7)
\]

\[
I_{λ2}(D, Z) \propto I_{02} \left( 1 - \sqrt{1 - NA^2} \right) e^{-ξ_2Z} \frac{D^2}{2} e^{-ξ_2Z} \quad \ldots \quad (8)
\]

\[
Z = \frac{1}{ξ_2 - ξ_1} \ln \left( \frac{k \cdot I_{02} \cdot I_{λ1}}{I_{01} \cdot I_{λ2}} \right) \quad \ldots \quad (9)
\]

3. Development of an Optical Apparatus

3.1. The Developed Apparatus and Optical System

In our wet-nanoscale optics laboratory, a real-time monitoring apparatus for multi-wavelength evanescent fields was developed to measure the 3D motion of an individual nanoparticle in an aqueous environment [12–14]. In this study, the developed apparatus for verifying the height $Z$ of nanoparticles was divided into two main parts: Part 1) optical microscopy system and Part 2) nanoparticle displacement control system, as shown in Fig. 5. For Part 1), the evanescent fields generated from the dual-
wavelength laser sources emitted light, which is scattered by the nanoparticles. Optical microscopy devices can accumulate scattering light intensities from the observed nanoparticles. An 8-bit color camera was used to detect the scattered light by the nanoparticles. For Part 2), the adhered nanoparticles on a spherical tip were connected to the piezoelectric actuator to control the nanoparticle displacement in the Z-direction. The scattered light of the nanoparticles and the piezoelectric displacement were recorded and displayed on a computer monitor in real-time.

3.2. Designing an Invisibility Spherical Tip

An invisible spherical tip is an essential aspect that could increase the effectiveness of the experimental verification, aiming to avoid signal noise from the spherical tip while observing the nanoparticle in water. Therefore, an optical adhesive (Norland Optical Adhesive: NOA1348, refractive index \( n = 1.348 \)) was selected to form the spherical tip because it has a refractive index close to that of water. However, the optical adhesive NOA1348 is an elastic material with a hardness of 30 Shore D, which can maintain the shape of the spherical tip but will be deformed after being compressed on the reference surface. The optical adhesive NOA1348 has a special ability to almost disappear when dipped into water, as shown in Fig. 6, which should be utilized to make the invisible spherical tip.

Besides, the influence of the spherical tip materials on nanoparticle observation will be studied using a simulation method to increase the effectiveness of our novel method. The finite-difference time-domain (FDTD) method is a powerful tool for modeling nanoscale optical systems and was proposed to simulate the effect of the spherical tip material. Different materials with a low refractive index (NOA1348, \( n = 1.348 \)) and high refractive index (glass, \( n = 1.52 \)) were simulated to compare the effects of the spherical tip. Furthermore, a nanoparticle without a spherical tip is the desired target used to compare the simulated results because it does not affect the spherical tip. Thus, the FDTD simulation conditions were divided into three cases: (1) nanoparticle without a spherical tip, (2) nanoparticle adhered to the spherical NOA1348 tip, and (3) nanoparticle adhered to the spherical glass tip, as shown in Fig. 7(a). The FDTD parameter settings between nanoparticle without a spherical tip and nanoparticle on a spherical tip were explained, as shown in Figs. 7(b) and (c), respectively.

The FDTD simulation results were divided into two main parts: the desired target (nanoparticle without the spherical tip) and affected from the spherical tip (nanoparticle adhered on the spherical tip), as shown in Fig. 8. For the nanoparticle without a spherical tip, the evanescent field propagation was created perfectly from the total internal reflection (TIR) of light, as shown in Fig. 8(a), which also could perfectly generate the scattering light from the nanoparticle, as shown in Fig. 8(b). For the nanoparticle adhered to the spherical NOA1348 tip, the evanescent field propagation was created almost perfectly close to the desired target, as shown in Fig. 8(c). Fig. 8(d) shows that the scattering light of the nanoparticle was higher than the desired target, slightly influenced by the spherical NOA1348 tip, which did not significantly affect the measurement method. For the nanoparticle adhered to the spherical glass tip, the evanescent field could not be created by total internal reflection because the light refracted passed through the nanoparticle to the sph-
A Novel Method for 3D Nanoscale Tracking of 100 nm Polystyrene Particles in Multi-Wavelength Evanescent Fields Microscopy

Fig. 7. The FDTD simulation method. (a) The FDTD conditions of three analysis cases. The FDTD simulation setup and parameters of (b) nanoparticle without spherical tip, and (c) nanoparticle on the spherical tip.

Fig. 8. The FDTD simulation results for analyzing the spherical tip material effect on a nanoparticle observation.

Fig. 9. The particle size distribution results using the dynamic light scattering (DLS) instrument from the repeated five measurements of polystyrene 100 nm particle samples.

3.3. Individual Nanoparticle Preparation

Polystyrene (PS) φ100±3 nm standard particles (Thermo Scientific™ 3100A Nanosphere™ Size Standards) were selected for 3D nanoscale tracking and verification of the experimental height because of the highly uniform particle size and high dispersity. For particle preparation, first, the PS 100 nm particles were dispersed in water using an ultrasonic machine for 30 min. Then, the nanoparticle size was determined using a dynamic light scattering (DLS) instrument. The particle size distribution result had a nominal mean diameter of 100.3 nm, as shown in Fig. 9. Subsequently, the nanoparticles were mixed in pure water within 0.001% solid to decrease the number of particles for higher single-particle dispersion.

3.4. Adhering Nanoparticles on the Spherical Tip

The procedure for nanoparticle adhesion on the spherical NOA1348 tip is illustrated in Fig. 10. Fig. 10(a) shows PS φ100 nm particles in 0.001% solid of 2 µL were dropped on the glass substrate. The nanoparticle samples were dried at room temperature (23°C) for 30 min, as depicted in Fig. 10(b). Fig. 10(c) shows that the dispersity of the dried nanoparticles was investigated using a dark-field microscope (Nikon Eclipse LV150N, 100x/NA0.9). The figure displays varied scattering intensities of the spherical glass tip, which instead occurred in the dark field (Fig. 8(e)). The scattered light from the nanoparticle almost diminished as the refracted light passed through the nanoparticle (Fig. 8(f)).

However, only the scattered light under the reference surface was considered and analyzed because an objective lens collected the light near the bottom area of the reference surface. According to the FDTD results, the scattered light from the particle between the desired target and particle on the spherical NOA1348 tip had almost the same intensity under the reference surface, as shown in Figs. 8(b) and (d). Therefore, the spherical NOA1348 tip can be used in the experiment because the scattering light from the nanoparticle can be distinguished near the desired target result, which does not affect our measuring method. On the other hand, the spherical glass tip with a high refractive index could not be used for our measuring method because the scattering light of the nanoparticle disappeared under the reference surface, as shown in Fig. 8(f).
nаночастиц. Низшие интенсивности были ассоциированы с single-nanoparticles, whereas the higher intensities were two or more nanoparticles that came close together, which evidently increased the scattering light intensity several times. As the distance between the two nanoparticles was lower than the lateral resolution limit \( \delta = 0.61 \lambda / NA \), which again depends on the NA of the objective lens and the wavelength of the light source in the microscope, only single nanoparticles were selected in the experiment. Then, the glass substrate with nanoparticle samples was prepared for the adhering process, and the spherical NOA1348 tip was installed with the piezoelectric actuator for precise movement in the \( Z \)-direction, as shown in Fig. 10(d). Fig. 10(e) illustrates that the spherical tip was moved down to the single nanoparticle dispersion on the glass substrate by the piezoelectric actuator. Then the spherical tip with the adhered nanoparticles was moved upward, completing the adhering process, as shown in Fig. 10(f).

### 4. Experimental Conditions and Setup

#### 4.1. Optical Apparatus Setting

The schematics in Fig. 11 represent the apparatus for optical multi-wavelength microscopy, which is an enlarged version of Fig. 5. Laser sources with wavelengths 642 and 450 nm (model: OXXIUS-IBX-642 and IBX-450) were employed. We combined the laser beams from two inputs into a single fiber using a two-wavelength combiner. The propagating laser beams were collimated using a fixed-focus collimator. The actual values of laser power for both wavelengths emitted from the collimator were established at 11 mW \( (I_{01} = I_{02}) \), which was measured using a laser power meter before the beam enters the plano-convex lens. The evanescent fields at the interface or the reference surface were generated by illuminating the laser beams to the plano-convex lens at an actual incident angle of 68° \( (i_1 = i_2) \), which is larger than the critical angle. The incident angle of the laser beam was controlled using a high-precision rotation mount. For verification, we measured laser power for both the lasers after TIR \( (I_{R1}, I_{R2}) \), which decreased to the same value 9.4 mW \( (I_{R1} = I_{R2}) \). Thus, we confirmed that the reflectance and transmittance ability of the reference surface material (plano-convex lens: BK7 glass) for both wavelengths were almost perfect, which is acceptable for verification purposes. The scattering light from nanoparticles is detected when the nanoparticles are moved in the evanescent fields using a piezoelectric actuator. The evanescent fields are perpendicular to the reference surface in the \( Z \)-direction, and the emitted light is scattered by the nanoparticles. An objective lens with NA (Nikon: 50x/N.A.0.45) collected scattering light intensities \( (I_{\lambda 1}, I_{\lambda 2}) \) from the nanoparticle. The scattering light intensities passed through the tube lens and focused on the CMOS image sensor with an 8-bit color camera. The detected scattering light intensities from the nanoparticles were then displayed on a computer monitor.

The experimental parameters and conditions of the optical system are listed in Table 1. The refractive index of each material indicated a small difference between both wavelengths \( (\lambda_1, \lambda_2) \), which should not have a signifi-
Table 1. The experimental parameters of the optical multi-wavelength evanescent fields microscopy.

<table>
<thead>
<tr>
<th>Laser Illumination</th>
<th>unit</th>
<th>$\lambda_1$</th>
<th>$\lambda_2$</th>
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<tr>
<td>-Wavelength: $A$</td>
<td>nm</td>
<td>642</td>
<td>450</td>
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<tr>
<td>-The measured power at incident: $I_{\lambda_1}/I_{\lambda_2}$</td>
<td>mW</td>
<td>11</td>
<td>11</td>
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<tr>
<td>-The measured power after TIR: $I_{\lambda_1}/I_{\lambda_2}$</td>
<td>mW</td>
<td>9.4</td>
<td>9.4</td>
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<tr>
<td>-Incident angle: $i_1 = i_2$</td>
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<table>
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<tr>
<th>Refractive index of materials</th>
<th>$n_1$</th>
<th>$n_2$</th>
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<tr>
<td>-Plano-convex lens (BK7 glass)</td>
<td>1.515</td>
<td>1.525</td>
</tr>
<tr>
<td>-Water</td>
<td>1.331</td>
<td>1.337</td>
</tr>
<tr>
<td>-Spherical NOA1348 tip: $n_{tip}$</td>
<td>1.348 (at 589 nm)</td>
<td></td>
</tr>
<tr>
<td>-Polystyrene particle: $n_{pol}$</td>
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<td>1.614</td>
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<table>
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<tr>
<td>-Total magnification</td>
<td>76x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Numerical aperture: NA</td>
<td></td>
<td>0.45</td>
<td></td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>CMOS Camera (8-bit color)</th>
<th>unit</th>
<th>$\lambda_1$</th>
<th>$\lambda_2$</th>
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<tr>
<td>-Relative response</td>
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<td>0.98</td>
<td>0.66</td>
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<tr>
<td>-Pixel size</td>
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<td></td>
</tr>
<tr>
<td>-Exposure time</td>
<td>ms</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>-Frame rate</td>
<td>fps</td>
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</tr>
<tr>
<td>-Recording images</td>
<td></td>
<td>Image/stroke</td>
<td>400</td>
</tr>
</tbody>
</table>

Fig. 12. Locations of the nanoparticles that were adhered on the spherical NOA1348 tip surface.

cant effect on the nanoparticle observation in our verification method. The total magnification of our optical microscopy system was approximately 76x, which was calibrated using a standard resolution target (USAF-1951/MIL-S-150A Standard Pattern). The CMOS camera sensitivity for both wavelengths was compensated using the relative response coefficients of 0.98 and 0.66.

4.2. Nanoparticle Displacement Control of Height $Z$

As the nanoparticles adhered to the spherical tip, three PS particles: particles A, B, and C, were located on the spherical tip surface, as shown in Fig. 12. A piezoelectric actuator with a strain-gauge feedback sensor (MESS-TEK: MPA-UA1S with controller: M-2510) was employed to control the height $Z$ of the adhered nanoparticles on the spherical tip. The piezoelectric actuator stroke length and repeatability were 16 $\mu$m and $\pm 1$ nm, respectively. In the experiment, the closed-loop system was used to control the nanoparticle displacement at a height $Z$ for every stroke of 20 nm until the nanoparticles touched the reference surface at a total of 10 strokes (from $\Delta z_{10}$ to $\Delta z_{10}$), as shown in Fig. 13. The observed nanoparticles were recorded in 400 images in each piezoelectric actuator stroke ($\Delta z_j$) at a frame rate of 50 fps with an exposure time of 5 ms using an 8-bit color camera. The input and displacement signals of the piezoelectric actuator were measured using the strain gauge feedback sensor during the experiment, as shown in Fig. 14.

5. Nanoparticle Tracking Method

5.1. The $X$, $Y$ Position Determination

The $X$ and $Y$ position tracking of the particles was analyzed by calculating the centroid of the scattering light intensity. Comparing the centroid of two sequence images of the scattered light from the nanoparticle is an analytically simple and computationally efficient method for tracking the $X$ and $Y$ positions of a single-nanoparticle motion [17, 18]. In this study, only the scattering light ($I_{\lambda_2}$) was used to precisely measure the $X$ and $Y$ positions because the scattering light intensity $I_{\lambda_2}$ has a higher contrast than $I_{\lambda_1}$. The centroid of the scattering light intensity on the center mark (white cross-line) from the tracked particle was used to determine the $X$ and $Y$ positions in each.
Fig. 15. Determination of the X and Y positions of the single-nanoparticle using the centroid of the scattering light intensities $I_{x2}$ from particle A.

Fig. 16. Evaluation of lateral resolution of our developed apparatus by measuring the polystyrene 100 nm particles adhered on the reference surface in the water.

frame accurately, as shown in Fig. 15. Furthermore, lateral ($X, Y$) resolution analysis is essential to evaluate the ability of the microscopy system to distinguish details, which is used to define the minimum distance between two distinct nanoparticles. Generally, the Abbe or Rayleigh criteria are widely used to determine the lateral resolution, whereas the Rayleigh criterion defines the resolution mathematically, as shown in Eq. (10).

$$\delta = \frac{0.61 \lambda}{NA}$$

where $\delta$ is the lateral resolution (the distance between the two particles), $\lambda$ is the wavelength of the laser, and $NA$ is the numerical aperture of the objective lens that collects the scattered light. The lateral resolution $\delta$ was approximately 610 nm, calculated using the Rayleigh criterion (where $\lambda = 450$ nm, $NA = 0.45$). However, the lateral resolution of our apparatus was approximately 500 nm, as measured from the Airy disks of scattering light intensity ($I_{x2}$) profiles (Fig. 16). This implies that our developed apparatus could distinguish the individual nanoparticles when the distance between two particles is greater than 500 nm.

5.2. Determination of Height $Z$

The height $Z$ is the longitudinal distance determined from the two-dimensional image of the scattered light intensities from the individual nanoparticles. The dual-wavelength scattering light image was split into two to integrate the scattering light intensity ($I_{x2}$) area of each wavelength ($\lambda_1 = 642$ nm and $\lambda_2 = 450$ nm), as shown in Fig. 17. The logarithmic ratios between the scattering light intensities ($I_{x1}, I_{x2}$) were substituted into Eq. (9) to calculate height $Z$ of each nanoparticle. The scattering light images also show the position shift for both wavelengths caused by the chromatic aberration (red at left and blue at right) but did not affect the height verification. However, the chromatic aberration can be improved by changing the objective lens type to Apochromat, and combining multiple wavelengths to the same position, which will be our future work.

6. Nanoparticle Tracking Results

6.1. 3D Nanoscale Tracking Results

According to the experiment, the three particles observed at different locations on the spherical tip were particles A, B, and C, which moved downward every 20 nm for each piezo stroke in a total of 10 strokes ($\Delta z_{s1} – \Delta z_{s10}$). Therefore, the 3D tracking results of the three nanoparticles on the spherical tip surface were plotted by combining the X, Y, and Z position results, as shown in Fig. 18.

6.2. The Verification Results of the Absolute Difference Height Between Nanoparticles

This study is focused on the height Z verification to evaluate the performance of our method by controlling the nanoparticle displacement. In the experiment, the height $Z$ of the nanoparticles was measured at 400 times per stroke displacement, in which the nanoparticles were
A Novel Method for 3D Nanoscale Tracking of 100 nm Polystyrene Particles in Multi-Wavelength Evanescent Fields Microscopy

Fig. 18. The 3D nanoscale tracking results of three nanoparticles. The height $Z$ results were projected on the wall (dark line). The scattering light images at some height $Z$ of particle A were displayed at the right-hand side.

Fig. 19. Typically measured results of nanoparticle displacement (of particle A) in each stroke, including the uncertainty ($2\sigma$ from 400 measurements).

Displaced vertically down to the reference surface with a stroke of 20 nm by a piezoelectric actuator. Fig. 19 shows typical height $Z$ results of particle A, including a measurement uncertainty (two times the standard deviation: $2\sigma$ from 400 measurements) in each stroke to verify the displacement identification of the height $Z$. The nanoparticle displacement could detect changes of less than $\pm 17$ nm ($2\sigma$), depending on the height from the reference surface. The measurement uncertainty was better when the nanoparticles were closer to the reference surface. Fig. 20 shows the compensated height $Z$ results of three nanoparticles in each stroke calculated using the average of the scattering light intensities from 400 recording images per stroke. According to the results, we found that the spherical tip starts to approach the reference surface no later than the fifth stroke, which is considered by the mostly unchanged scattering lights of particle B. Then, particle A should have touched the reference surface at the ninth stroke because the scattering light intensities ($I_{\lambda_1}, I_{\lambda_2}$) of particle A were mostly unchanged after this stroke, which was caused by the spherical tip touching the reference surface. Therefore, the $k$ value was 1.14, which can be calculated by substituting the height $Z$ equal to zero into Eq. (9). Subsequently, in the experiment, the nanoparticles continued to move down, even when the spherical tip touched the reference surface owing to the elastic deformation of the tip. The elastic spherical tip was deformed by a rigid reference surface with an upper piezoelectric load. According to the 3D tracking results after the tip deformation in Fig. 18, particles A and C had position shifts in the $X$ and $Y$ directions but had no evident shift in the height $Z$ until the eighth stroke, as shown in Fig. 20. However, we could not gradually withdraw the nanoparticles because the deformed tip adhered to the reference surface, and the tip jumped suddenly after withdrawing for a while. Hence, the nanoparticle height $Z$ could not be successfully observed.

Therefore, the compensated heights $Z$ analyzed the absolute difference in height between nanoparticles, which corresponded to the feedback displacement control of the piezoelectric actuator. Fig. 21 shows the measurement parameters of the three nanoparticles that were used to analyze the absolute difference in height between nanoparticles, where $\Delta Z_{AB}, \Delta Z_{CA}$ are the absolute difference height between particles A and B and particles C and A, re-

Fig. 20. The compensated height $Z$ results of three nanoparticles were plotted with the number of stroke.

Fig. 21. The measurement parameters for evaluating the absolute difference height between nanoparticles.
respectively. The results of the absolute difference height between nanoparticles ($\Delta Z_{AB}$, $\Delta Z_{CA}$) for each stroke are plotted in the graph (Fig. 22). However, stroke number 10 was not plotted and analyzed because the spherical tip had already touched the reference surface. Fig. 22 also shows that the measurement repeatability of the absolute difference height between nanoparticles was approximately less than ±16 nm.

7. Conclusion

Multi-wavelength evanescent field microscopy has been proposed to measure the 3D nanoscale tracking of a near-surface individual nanoparticle in a wet process. This study focused on the experimental verification of the measured longitudinal height $Z$ of a nanoparticle in water. Polystyrene standard nanoparticles ($\phi 100\pm3$ nm) were used because of their high uniform size and high dispersity. The individual PS nanoparticles adhered to the surface of the spherical tip. The spherical tip was made from an elastic material with a refractive index $n = 1.348$ close to that of water ($n = 1.33$), which was accepted to be invisible when dipped into the water based on the experimental and simulation results. The experiments were performed by employing a piezoelectric actuator to control the displacement of the adhered nanoparticles on the spherical tip in the Z-direction. The developed apparatus could detect the scattering light intensities from nanoparticles using an 8-bit color camera at a frame rate higher than 50 frame per second for an exposure time of 5 ms. The penetration height measurability range was approximately 250 nm from the reference surface, which can be validated by using the displacement feedback data from the piezoelectric actuator. After compensating the height $Z$ (with an uncertainty of $2\sigma \approx 17$ nm), we found that the measurement repeatability of the absolute difference height $\Delta Z$ between nanoparticles could be less than that of ±16 nm.

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A Novel Method for 3D Nanoscale Tracking of 100 nm Polystyrene Particles in Multi-Wavelength Evanescent Fields Microscopy

Name: Aran Blattler
Affiliation: Advanced Mechanical Division, Graduate School of Computer Science and System Engineering, Kyushu Institute of Technology
Address: 680-4 Kawazu, Iizuka, Fukuoka 820-8502, Japan
Brief Biographical History:
1985-1989 Graduated from Nagoya Institute of Technology
1989-1991 Rotary Yoneyama Scholarship Grantee
1992-1993 Master Student, Kyushu Institute of Technology
2003-2005 Ph.D. Student, Kyushu Institute of Technology
2005-2006 Research Associate, Advanced Mold and Die Technology Center, Kyushu Institute of Technology
2006- Associate Professor, Kyushu Institute of Technology
2010- Professor, Department of Intelligent and Control Systems, Faculty of Computer Science and Systems Engineering, Kyushu Institute of Technology
Main Works:
• Laser applied precision engineering
• R&D for CMP process technology
Membership in Academic Societies:
• Japan Society for Precision Engineering (JSPE)

Name: Panart Khajornrungruang
Affiliation: Associate Professor, Department of Intelligent and Control Systems, Faculty of Computer Science and Systems Engineering, Kyushu Institute of Technology
Address: 680-4 Kawazu, Iizuka, Fukuoka 820-8502, Japan
Brief Biographical History:
2000-2002 Graduated from Osaka University
2006-2007 Rotary Yoneyama Scholarship Grantee
2006-2007 Ph.D. Student, Kyushu Institute of Technology
2007- Assistant Professor, Kyushu Institute of Technology
2010- Associate Professor, Department of Mechanical Information Science and Technology, Faculty of Science and Engineering, Kyushu Institute of Technology
2013- Associate Professor, Kyushu Institute of Technology
2017- Master Student, Kyushu Institute of Technology
Main Works:
• Laser applied precision engineering
• Live 3D nanoparticles observation
• R&D for CMP process technology
Membership in Academic Societies:
• Japan Society for Precision Engineering (JSPE)
• Japan Society of Mechanical Engineers (JSME)

Name: Keisuke Suzuki
Affiliation: Professor, Department of Intelligent and Control Systems, Faculty of Computer Science and Systems Engineering, Kyushu Institute of Technology
Address: 680-4 Kawazu, Iizuka, Fukuoka 820-8502, Japan
Brief Biographical History:
1984-1989 Graduated from Osaka University
1989-1991 Rotary Yoneyama Scholarship Grantee
1992-1993 Master Student, Kyushu Institute of Technology
2003-2005 Ph.D. Student, Kyushu Institute of Technology
2005-2006 Research Associate, Advanced Mold and Die Technology Center, Kyushu Institute of Technology
2006- Associate Professor, Kyushu Institute of Technology
2010- Professor, Department of Intelligent and Control Systems, Faculty of Computer Science and Systems Engineering, Kyushu Institute of Technology
Main Works:
• Chemical mechanical polishing
• Development semiconductor process
• R&D for nano materials
• Advanced processing technology
Membership in Academic Societies:
• Japan Society for Precision Engineering (JSPE)
• Japan Society of Applied Physics (JSAP)
• Japan Society of Mechanical Engineers (JSME)

Name: Soraya Saenna
Affiliation: Department of Interdisciplinary Informatics, Graduate School of Computer Science and Systems Engineering, Kyushu Institute of Technology
Address: 680-4 Kawazu, Iizuka, Fukuoka 820-8502, Japan
Brief Biographical History:
2009-2012 M.Sc. Student, Graduate School of Computer Science and Systems Engineering, Kyushu Institute of Technology
2012-2014 Ph.D. Student, Graduate School of Computer Science and Systems Engineering, Kyushu Institute of Technology
2019- Master Student, Kyushu Institute of Technology
Main Works:
• Simulation on 3D nanoparticles tracking
• Finite-difference time-domain (FDTD) method
Membership in Academic Societies:
• Japan Society for Precision Engineering (JSPE)