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Generation and Control of 3D Standing Wave Illumination for Wide-Field High-Resolution 3D Microscopic Measurement

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Microscopic imaging is convenient, nondestructive, and has a high-throughput performance and compatibility with a number of applications. However, the spatial resolution of conventional light microscopy is limited to wavelength scale and the depth of field is extremely shallow; hence, it is difficult to obtain detailed 3D spatial data of the object to be measured. In this paper, we propose a new technique for generating and controlling 3D standing wave illumination based on the 3D interference of multiple laser beams. The proposed technique has possibility to provide lateral and axial resolution improvement as well as a wide 3D field of view. The spatial configuration of the interference beams was theoretically examined and the optimal incident angle of the multiple beams was confirmed. Numerical simulations were carried out and confirmed the generation of 3D standing wave illumination and spatial control of the illumination by using the phase shift of incident beams. Furthermore, we develop an experimental apparatus to demonstrate the generation of 3D standing wave illumination by four beam interference and spatial control with a piezoelectric transducer. Finally, basic experiments were performed using nanospheres to verify the generation, spatial intervals, and controllability of the phase shift of 3D standing wave illumination.

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NOMENCLATURE

3D = three dimensional
NA = numerical aperture
FDTD = finite difference time domain
PZT = piezoelectric transducer
CCD = charge-coupled device
PSL = polystyrene latex

1. Introduction

Digital microscopic imaging can be utilized for a number of applications by combining image and signal processing techniques, e.g., reviewing patterned semiconductor wafers, visualizing printed circuit boards, inspecting micrometer-scale industrial components, and medical and biological examinations. Numerous industries now not only require improvement in the lateral resolution but also the axial resolution of the images, and the imaging process needs to measure features of the object in three dimensions nondestructively at high resolution without compromising inspection speed. This is especially important since defect detection is becoming an increasingly challenging task with the shrinking size and complicated structures of the samples.[1] Inspections are conventionally performed by microscopic imaging using either an optical or electron beam method. Scanning electron beam microscopy does possess nanoscale resolution, but it is a destructive method and not compatible with wide-area inspections due to its low throughput performance. On the other hand, optical microscopy is a commonly used imaging technique and easy to implement in various situations. However, the inability of optical microscopy to handle the continuous miniaturization of components has become a major issue, because the lateral and axial resolution of the method is limited by the wavelength at which diffraction occurs. In this study, we concentrate on the optical method because it is nondestructive and ensures a high throughput. The optical method can also potentially provide a higher resolution on the subwavelength scale.

Generally, in microscopic imaging, the lateral resolution of an optical system is diffraction limited; it is determined by wavelength of the incident light and the objective lens numerical aperture (NA). The resolution of the optical system will improve with an increase in NA and decrease with increasing wavelength. In the practical use of a microscope, however, it is difficult to decrease the wavelength to the ultraviolet region. Additionally, the upper limit of NA in dry conditions is 1.0. Furthermore, classical wide-field optical microscopy has another limit in the axial imaging direction (only relevant in three-dimensional (3D) imaging) in which complete information of specimen is not obtained. This is known as the missing cone problem;[2] in a sequence of 2D images with different foci, there is not only in-focus information from the corresponding section of the sample but also out-of-focus blur from all other sections.

In order to overcome the diffraction limit and the missing cone problem in 3D microscopic imaging, we propose applying 3D structured illumination to digital microscopic imaging. In structured illumination microscopy,[3] the lateral spatial resolution can be improved by a factor of two compared with conventional optical microscopy. This improvement is based on the moiré effect in optical interference such that the high spatial frequency information of the structured illumination and specimen can pass the spatial frequency band of the optical microscopy system. It is possible to apply this technique to the inspection of industrial components, in which the spatial control of the illumination and multi-image reconstruction deliver optimal sensitivity and a higher signal-to-noise ratio for critical defect detection at the subwavelength scale. The optical measurement system used in this study is characterized by specific properties such as nondestructiveness, high resolution, and high throughput. Theoretical and experimental verifications reveal that the use of structured light illumination together with successive approximation (which provides an extrapolation effect) results in the resolution of the proposed method exceeding the diffraction limit in optical microscopic imaging.[4] Furthermore, subpixel spatial shifts of the structured illumination can be reflected in the final reconstruction. Thus, the proposed method can possibly improve the resolving power not only beyond the diffraction limit but also beyond the limit determined by the spatial sampling.[5]

2. Proposal of Simultaneous Improvement of the Lateral and Axial resolution by Truly 3D Structured Illumination

The missing cone problem, however, still remains in previous works of the 2D structured illumination imaging. In addition, structured illumination has been used to provide axial optical sectioning, but in this case, it does not provide much improvement in the lateral resolution.[6] This tradeoff can be avoided by using 3D structured illumination generated from a diffraction grating and an objective lens with an extremely high NA,[7] but the 3D structured illumination field produced by this previous method is too localized to be applied for industrial inspections, because there is a tradeoff between spatial resolution and the field of view. In addition, the specimen stage must be scanned axially for optical sectioning because the axial pitch of the 3D structured illumination is wider than the diffraction limit. Moreover, generated 3D structured illumination is basically 2D, so several kinds of specimen stage rotations are needed to 3D high spatial frequency information. There are a lot of difficulties in the previous works in terms of practical applications.

Hence, we propose a new technique to generate and control truly 3D structured illumination which is 3D standing wave illumination based on the 3D interference of multiple laser beams. This technique provides simultaneous improvement of lateral and axial resolution even in the low NA conditions, so a wide 3D field of view and a very long working distance are obtained. Although high-resolution 3D imaging with this technique is confined to the observation of specimens with optical transmittance, 3D high spatial frequency information is obtained by only laser phase control without stage rotation and axial stage driving.

3. Methodology for 3D Standing Wave Illumination Generation

3.1 3D Standing Wave Illumination Based on Multiple Laser Beam Interference

In order to improve the 3D spatial resolution of optical microscopy without compromising the imaging field of view, we propose a 3D structured illumination generation technique that is based on standing wave illumination with multiple laser beam interference. An example of the spatial configuration of the multiple laser beams for 3D standing wave illumination is shown in Fig. 1. In this configuration, four laser beams enter the microscopic field and interfere to generate the standing waves. The phase difference between laser beams #0 and #1 corresponds to the spatial displacement of the 3D standing wave illumination in the X direction, the phase difference between beams #2 and #3 displaces the 3D standing wave illumination in the X direction, and the spatial position of the 3D standing wave illumination in the Z direction is controlled by changing the phase of beams #0 and #1 simultaneously.

3.2 Optimization of the Incident Angle in Four Laser Beam Interference

In structured illumination microscopy, the spatial pitch of the illumination intensity distribution, which corresponds to the peak-to-peak distance of the interference fringes, is an important factor in improving the spatial resolution. The spatial pitch is determined by the

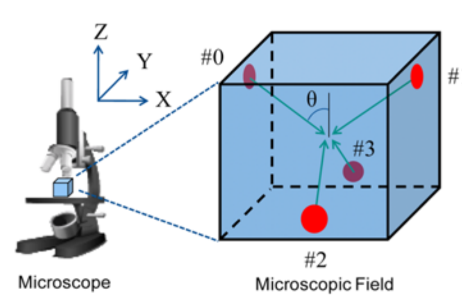

Fig. 1 Spatial configuration of the incident beams in the microscopic field for 3D standing wave illumination based on four beam interference. The incident vectors of the beams are $(x, y, z) = (\sin\theta, 0, -\cos\theta)$ for beam #0, $(-\sin\theta, 0, -\cos\theta)$ for beam #1, $(0, \sin\theta, \cos\theta)$ for beam #2, and $(0, -\sin\theta, \cos\theta)$ for beam #3

incident angle of four laser beams in the proposed configuration. Hence, the incident angle can be optimized for the isotropic and periodic intensity distribution of the 3D standing wave illumination with high spatial frequency.

The intensity distribution L^2 of the n-beam interference as a function of the position is described as

$$L^2 = \left|\sum_{i=0}^{n-1} e^{-j\vec{k}_i\cdot\vec{r}}\right|^2 = n + 2\sum_{i>k}\cos(\phi_i - \phi_k),\ \phi_i = \vec{k}_i\cdot\vec{r} \tag{1}$$

where $\vec{k}$ is the wave number vector and $\vec{r}$ is a position vector.

For four beam interference as shown in Fig. 1, the incident vector of beam #0 is (sinθ, 0, -cosθ), and is (-sinθ, 0, -cosθ) for beam #1, (0, sinθ, cosθ) for beam #2, and (0, -sinθ, cosθ) for beam #3, where θ is the incident angle. Therefore, the intensity distribution L^2 as a function of position x, y, and z in this case can be expressed as

$$\begin{aligned} L^2 = 4 + 2[&\cos\left\{\frac{2\pi}{\lambda}(2x\sin\theta)\right\} + \cos\left\{\frac{2\pi}{\lambda}(2y\sin\theta)\right\} \\ &+\cos\left\{\frac{2\pi}{\lambda}(x\sin\theta + y\sin\theta + 2z\cos\theta)\right\} \\ &+\cos\left\{\frac{2\pi}{\lambda}(x\sin\theta + y\sin\theta - 2z\cos\theta)\right\} \\ &+\cos\left\{\frac{2\pi}{\lambda}(x\sin\theta - y\sin\theta - 2z\cos\theta)\right\} \\ &+\cos\left\{\frac{2\pi}{\lambda}(x\sin\theta - y\sin\theta + 2z\cos\theta)\right\} \end{aligned} \tag{2}$$

where λ is wavelength of the incident laser beam. In our proposal, the four laser beam sources are placed with the high symmetry for uniformly periodic intensity distribution of the standing wave illumination. The positions of the sources #0, #1, #2 and #3 are shown in Fig. 1. The uniformly periodic intensity distribution is obtained when the laser beam propagates from a vertex to the median point of a regular tetrahedron.[8] The optimal incident angle under these conditions is approximately 54.7°. It is difficult, however, to implement a regular tetrahedron configuration due to the structural problems of the microscopic imaging system, e.g. interference of laser beams with objective lens. Hence, we examined the optimal incident angle for the configuration shown in Fig. 1. The spatial pitch of the 3D standing wave illumination is calculated by partial differentiation of Eq. (2) with respect to the positions x, y, and z and then obtaining the extreme value. The distance between the extreme values corresponds to the spatial pitch of the standing wave illumination. By this analysis, we found that the spatial pitch of the 3D standing wave illumination in the X and Y directions is equal to that in Z direction when the incident angle is approximately 63.4°. The corresponding spatial pitch in this case is 297 nm when the wavelength λ is 532 nm.

4. Numerical Simulation for 3D Standing Wave Illumination

3D multiple beam interference is a complicated phenomenon and the spatial pitch of the standing wave illumination is on the sub-wavelength scale, and thus, not observed. Therefore, we carried out numerical simulations based on the finite difference time domain (FDTD) method of the generation of the 3D standing wave illumination in advance of the experimental verifications.

Simulation models were designed according to the spatial configuration of the laser beams shown in Fig. 1. 3D FDTD simulations consume a lot of memory in a computer, because billions of elements are needed e.g. 10 nm × 10 nm × 10 nm element size in 10 mm × 10 mm × 10 mm region. Hence, the simulation models were designed assuming spatial periodicity for saving the memory. The simulation parameters are listed in Table 1. The laser beam was assumed to be a continuous wave with an incident angle of 63.4°, which provides the isotropic and periodic intensity distribution of the 3D standing wave illumination. It is important to control the polarization of the laser beam for a high contrast illumination intensity distribution. The laser beam in this simulation is assumed to be linear and p-polarized in the direction parallel to the incidence plane.

The resulting intensity distribution of the 3D standing wave illumination is illustrated in Fig. 2. The cross-sectional intensity distributions in the X-Y and X-Z planes are shown in Fig. 3. The isotropic, periodic intensity distribution with a spatial pitch of approximately 300 nm can be confirmed in Fig. 3. Additionally, we could control the spatial position of this distribution independently in all directions. Thus, 3D standing wave illumination was generated by the proposed method and the spatial pitch corresponds to that of the theoretical analysis. Consequently, we can generate and control the 3D standing wave illumination to improve the spatial resolution of the optical microscopic imaging.

Table 1 3D FDTD simulation parameters

Calculation area (side length)	5 μm
Grid size (side length)	50 nm
Beam diameter of laser source (continuous wave)	2 μm
Wavelength λ of laser source	532 nm
Incident angle θ	63.4°

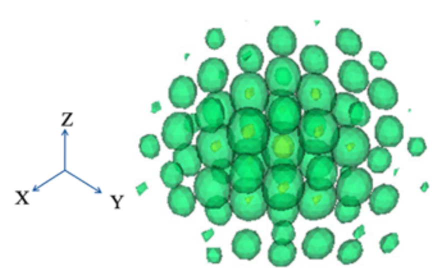

Fig. 2 Visualization of 3D standing wave illumination based on four beam interference calculated by the FDTD method

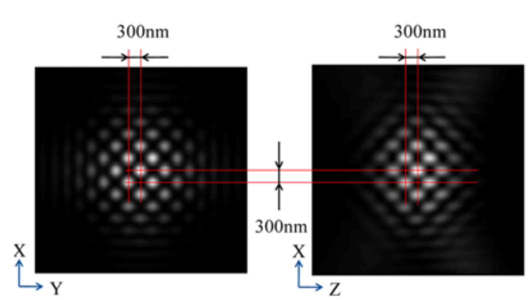

Fig. 3 Cross-sectional intensity distribution of 3D standing wave illumination with optimized multibeam incident angle

5. Experiment for Generation and Control of 3D Standing Wave Illumination

In general structured illumination microscopy, it is extremely important for resolution improvement to generate and control the structured illumination with modulation periodicity and certain spatial frequency of intensity distribution, and certain phase shift in the standing wave illumination for multiple image reconstruction. Therefore, the standing wave illumination was verified experimentally by measuring the modulations of the generated illumination.

5.1 Four Beam Interference Optical System Design

A schematic layout of the designed system is shown in Fig. 4 (top and total view) and in Fig. 5 (side view around the microscopic field). In Fig. 4, the linearly polarized laser source is divided into four beams using three beam splitters and is incident on the microscopic field through mirrors and beam steering components. The polarization direction is controlled with half-wavelength plates to maximize the contrast of the intensity distribution. The incident beams #0 and #1 of Fig. 1 correspond to the beams shown in Fig. 5(a) and beams #2 and #3 correspond to the beams in Fig. 5(b). The phases of beams #2 and #3 are shifted with the piezoelectric transducer (PZT) for spatial control of the 3D standing wave illumination.

5.2 Experimental verification of 3D Standing Wave illumination generation

The developed apparatus (Fig. 6) was used for generating the 3D standing wave illumination for imaging a polystyrene latex (PSL) nanosphere sample. Table 2 lists the conditions of the illumination and imaging optics of the experiments. The conditions are similar to those of the FDTD simulations. In the experiments, the laser beam diameter was approximately 1 mm so that the 3D standing wave illumination covered a wider microscopic field than that of the simulations. The spatial pitch of the 3D standing wave illumination generated by the proposed method is smaller than the diffraction limit of the optical microscope, so it is impossible to visualize the 3D standing wave illumination via the typical light microscopy.

The PSL sample consisted of standard nanospheres dispersed on a microscopic slide. The diameter of each sphere was 200 nm, and the spatial sampling of the imaging was approximately 65 nm, so the image intensity of the sphere was limited to the scattered light intensity. Therefore, standing wave illumination can be confirmed by measuring the modulations in the intensity of the sphere image during spatial shifts of the standing wave illumination.

In the experiments, spatial shifts in two directions (X and Z; Y is almost the same as X) of the generated 3D standing wave illumination and three interference conditions (four beam, two beam, and uniform illumination which is equivalent to one beam) were used to confirm the 3D standing wave illumination. In the case of four beam interference, if 3D standing wave illumination is generated, the image of a PSL nanospheres will be modulated in both the X and Z directions. On the other hand, in the case of two beam interference (beams #2 and #3), if 3D standing wave

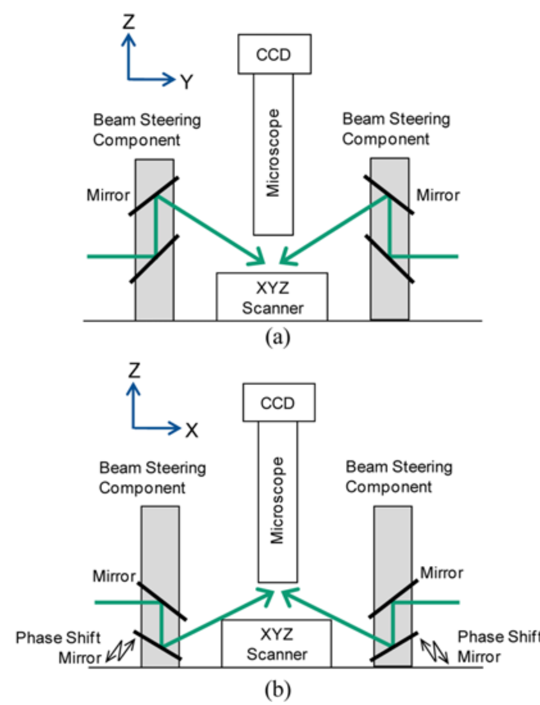

Fig. 5 Schematic design (side view) of the experimental apparatus around the microscopic field. Ray diagrams for (a) incident beams #0 and #1, and (b) incident beams #2 and #3 with a phase shift mirror for spatial control of the 3D standing wave illumination

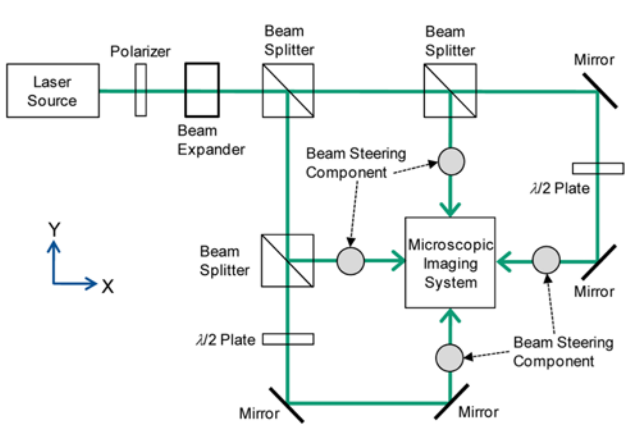

Fig. 4 Schematic design (top view) of the experimental apparatus for four beam interference

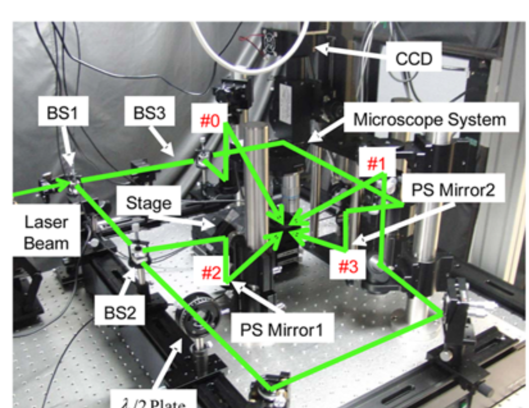

Fig. 6 Developed apparatus for 3D standing wave illumination generation

illumination is generated, the image of a PSL sphere will be modulated only in the X direction. There should be no image modulation at all in the uniform illumination case.

The relationships between the spatial shifts of the 3D standing wave illumination in the X direction and the intensity of the PSL sphere image are shown in Fig. 7 and Fig. 8. These figures correspond to the four beam and two beam interference cases, respectively. Image modulation with spatial shifts in the Z direction for all interference cases are shown in Fig. 9. Although the image intensity data contains some noise, such as speckles, optical fluctuations, and thermal drifts, the two beam and four beam interference images were modulated periodically in the X direction experiments. In the Z direction experiments, periodic modulation was confirmed only for the four beam interference image. The periodic modulation is fitted by sinusoidal curve to calculate the approximate phase of the standing wave illumination, in which the phase corresponds to the spatial position of the standing wave illumination. The illumination noise was present in Fig. 7, Fig. 8 and Fig. 9, where the generated interference fringe were not completely periodic because the multiple beams didn't have homogeneous power, polarization and spatial stability. Although the less difference are ideal, the illumination noise can be decreased using the averaging effect of multiple image reconstruction for resolution improvement. Hence, these results suggest that 3D standing wave illumination was generated and could be controlled in three dimensions.

Table 2 Experimental conditions

Wavelength λ of laser source (continuous wave)	532 nm
Beam Diameter @1/e^2 (expanded and collimated)	1 mm
Incident angle θ	63.4^o
Numerical aperture (NA) of objective	0.55
Magnification of imaging optics	100
Spatial sampling pitch of CCD	6.45 μm
Positioning resolution of PZT for phase shift	0.1 nm

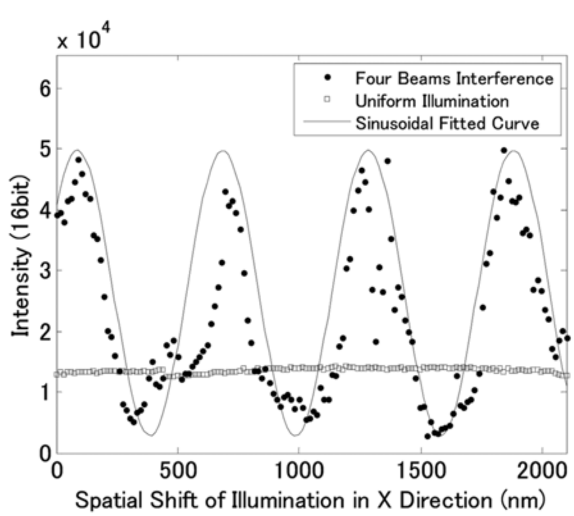

Fig. 7 Relationship between the spatial shift of the standing wave illumination in the X direction and the image intensity of a PSL sphere in the four beam interference and uniform illumination cases

6. Conclusions

We proposed a new technique to generate and control 3D standing wave illumination based on the 3D interference of multiple laser beams. This technique provides lateral and axial resolution improvement, and a wide 3D field of view for optical microscopic imaging. In this paper, the spatial configuration of the multiple beams was theoretically examined and the optimal incident angle of the multiple beams was confirmed. As a result of FDTD simulations, we confirmed the generation of 3D standing wave illumination and spatial control of the illumination by using the phase shift of the incident beams. Furthermore, we developed an experimental apparatus for generating the 3D standing wave illumination. In the developed apparatus, 3D standing wave illumination was generated by four beam interference and was spatially controlled. Finally, we performed basic experiments using PSL standard nanospheres to verify the generation and controllability of the phase shift of 3D standing wave illumination.

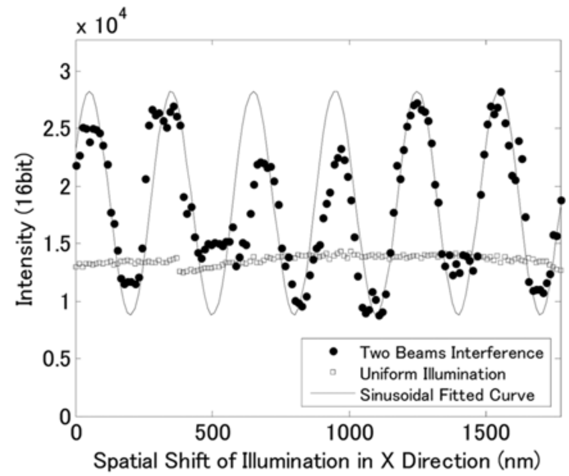

Fig. 8 Relationship between the spatial shift of the standing wave illumination in the X direction and the image intensity of a PSL sphere in the two beam interference and uniform illumination cases

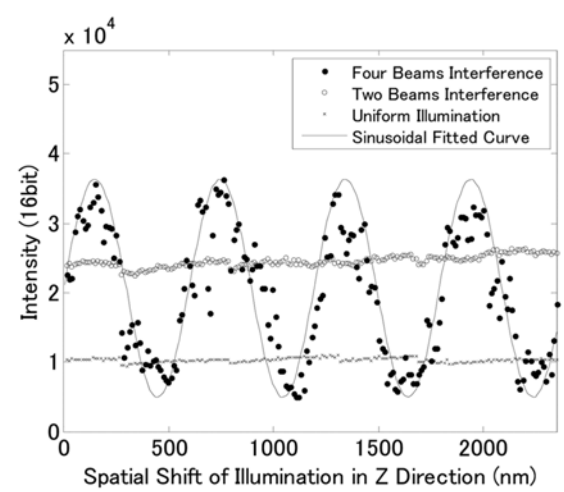

Fig. 9 Relationship between the spatial shift of the standing wave illumination in the Z direction and the image intensity of a PSL sphere in the four beam interference, two beam interference, and uniform illumination cases

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