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High contrast measurement of nanoparticle with polarization interferometric nonlinear confocal microscope

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ABSTRACT

Polarization interferometric nonlinear confocal microscope has been developed to observe a submicron size object in high contrast. The microscope succeeded in resolving the inside of a 200-nm-diameter polymeric nanoparticle. According to CTF (contrast transfer function) measurement and three-dimensional imaging with the microscope, the best spatial resolution for the microscope is 10 nm.

KEYWORDS

Confocal microscope; CTF; nanoparticle; scattering

Introduction

High contrast measurement methods for submicron object have been developed. These methods can be useful for many optical and medical applications [1–3]. Recently, an optical method invented in our laboratory has been expected to analyze a single nanoparticle of drug delivery system (DDS) [4].

In this letter, we propose polarization interferometric nonlinear confocal microscope, which observes the dielectric nanoparticle under the diffraction limit even with continuous wave (CW) laser [5]. Also, the microscope has an advantage that fluorescent probes are unnecessary when scanning the object.

CTF (Contrast Transfer Function) measurement and three-dimensional measurement of the single pseudo-DDS nanoparticle inhomogeneously doped with chromophores were performed to evaluate the resolution of the microscope proposed.

Experimental and theorical backgrounds

The polarization interferometric nonlinear confocal microscope proposed increases the CTF with images. Two fundamental techniques enhancing CTF are mentioned below.

In the first step, the microscope detects nonlinearFig. 1scattering near a focal point. With a focused illuminating probe beam, third-order nonlinear polarization $\mathbf{P}^{(3)}$ is induced in the center spot of the airy disk. The nonlinear polarization gives rise to an increase in a back scattered confocal signal. Consequently, the scattered light intensity increases, then the microscope improves the CTF of images.

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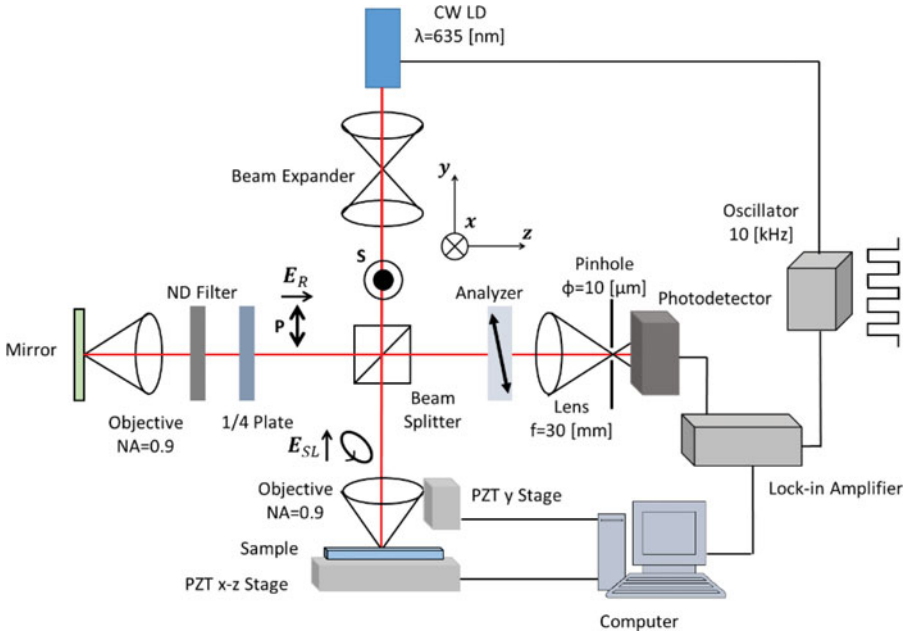

Figure 1. A schematic of the polarization interferometric nonlinear confocal microscope.

In the second step, the polarization interferometer equipped with the confocal microscope suppresses a bias electric field $\mathbf{E}_{SL}$. The electric field is previously set to zero. The adjustment of the transmission axis of the analyzer makes two electric fields, $\mathbf{E}_{SL}$ and $\mathbf{E}_R$, cancel each other, where $\mathbf{E}_R$ is the y-polarized electric field of a reference beam. Now, let us take the special case where high intensity light with x-polarization is incident on a sample and both field amplitudes are approximately equal, $|E_{SL}| \cong |E_R|$ . . In this case, the output light intensity is given by

$$\begin{aligned} |E_O(\theta = -\pi/4)|^2 &= |E_x(\theta = -\pi/4)|^2 + \left|E_y(\theta = -\pi/4)\right|^2 \\ &= |E_{SL}|^2 \left(1 - cos\Psi + sin\Psi cos\delta - \frac{1}{2} sin2\Psi cos\delta\right) \end{aligned} \quad (1)$$

Here, Ψ is an azimuth angle of the principal axis of the elliptically polarized light, and δ is a phase difference of the elliptically polarized light. With the polarization interferometer, no output signal of $|E_O(\theta = -\pi/4)|^2$ emerged from the background region. Hence, the polarization interferometer plays an important role in enhancing CTF with images.

Experiment

A schematic of the polarization interferometric nonlinear confocal microscope, which has two functions mentioned in the previous section, is shown in Fig. 3. A CW laser diode (LD) emitting at 635 nm is employed. The LD's output beam being S-polarized is split into two beams. One beam strikes a fixed mirror and the other collides with a sample. The reflected beam as being P-polarized and the scattered light as being elliptically polarized emerge, respectively. The recombined beams which travel through two different paths interfere on the analyzer. The microscope detects the polarization interferometric output light through the analyzer.

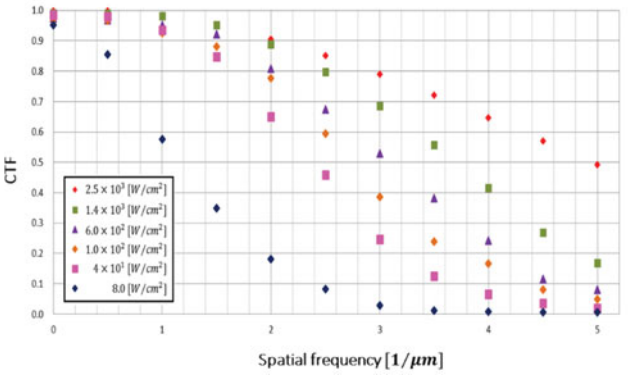

Figure 2. CTF curves based on the data measured with polarization interferometric nonlinear confocal microscope.

In our study, a sample observed under the microscope is an inhomogeneous anisotropic medium. The sample is an inhomogeneously chromophore-doped polystyrene particle (200 nm diameter). In this measurement, a thin sample ($\alpha_0 L = 0.45$) was employed, where α_0 is the absorption coefficient and L is the thickness of the sample.

Results and discussion

Following two measurements of the sample mentioned previously are factors determining the resolving power of the polarization interferometric nonlinear confocal microscope.

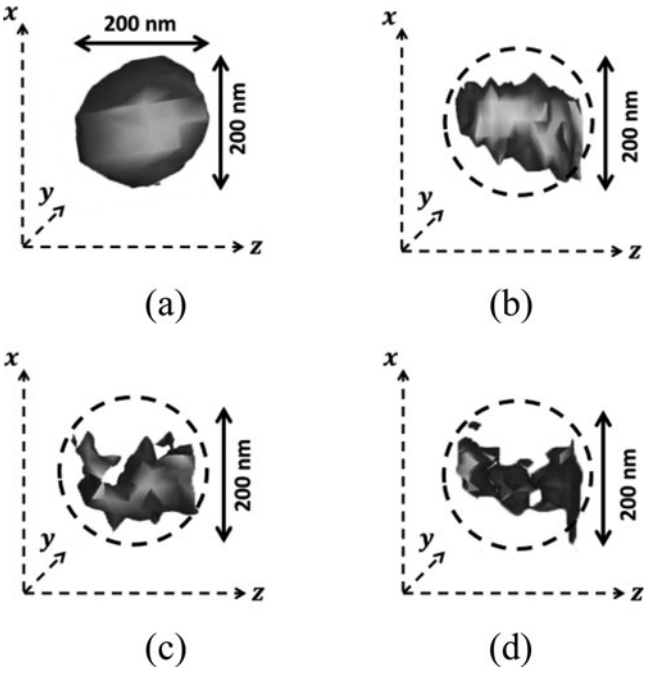

Figure 3. Three-dimensional images of a single nanoparticle: (a) surface image with conventional confocal microscope ($I = 1.0$ W/cm^2), (b) internal image with polarization interferometric linear confocal microscope ($I = 8.0$ W/cm^2), and (c)-(d) internal images with polarization interferometric nonlinear confocal microscope($I = 1.0\times10^2$ W/cm^2 and $I = 2.5\times10^3$ W/cm^2).

Firstly, CTF measurement of scattered signals along a straight line passing through center points of two nanoparticles, which are separated by a certain distance, determines the in-plane resolution of the microscope. CTF curves are depicted in Fig. 2. Here, spatial frequency is defined as the reciprocal of the distance between centers of two nanoparticles. According to Fig. 2, the in-plane resolution depends on the incident light intensity.

Secondly, three-dimensional measurement of a single nanoparticle determines the spatial resolution of the microscope. Conventional confocal microscope has poor spatial resolution, which only gives a surface image of a single nanoparticle (see Fig. 3(a)). On the other hand, the microscope proposed has better spatial resolution than conventional confocal microscope. The spatial resolution of the microscope gives images of the distribution of chromophores being distributed (see Fig. 3(b), (c) and (d)). These images reveal that the spatial resolution of the microscope depends on the incident light intensity. The microscope reaches 10 nm spatial resolution measured at the incident light intensity ($I = 2.5 \times 10^3$ W/cm^2).

Conclusion

From the results, we demonstrated that the resolving power of the polarization interferometric nonlinear confocal microscope is sufficient to observe a submicron object in high contrast.

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