

# Experimental verification of super-resolution microscopy using standing evanescent light with image retrieval

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## Abstract

The lateral resolution improvement of super-resolution microscopy using standing evanescent light with image retrieval is experimentally verified by detecting the standard fluorescent particles with the developed microscope system having standing evanescent light illumination shift control unit.

## 1 Introduction

Optical microscopy is still a useful metrology technique to observe a magnified image. Because light has intrinsic characteristics of low energy and spatial transmission, no damage is incurred to samples and high throughput is achievable by remote imaging. The major limitation of optical microscopy is the diffraction limit in resolution, which is about half the wavelength. Since measurement objects have been miniaturized in Semiconductor industry and Biotech industry, new optical measurement methods with high resolution and various applications have been strongly required. Then, we propose a super-resolution microscopy that employs a combination of standing evanescent light and an image distribution retrieval algorithm with successive approximation [1].

## 2 Super-resolution microscopy using standing evanescent light with image retrieval

Figure 1 shows a schematic diagram of our proposed method. Standing evanescent light is formed on the interface of total internal reflection by superposition of incident light and counter-propagating incident light. The standing evanescent light has high-frequency modulation in the lateral direction, where the peak-to-peak distance  $T$  is about half the wavelength (several hundred nanometers). The illuminated sample's distribution is magnified and detected through far-field imaging optics, which is

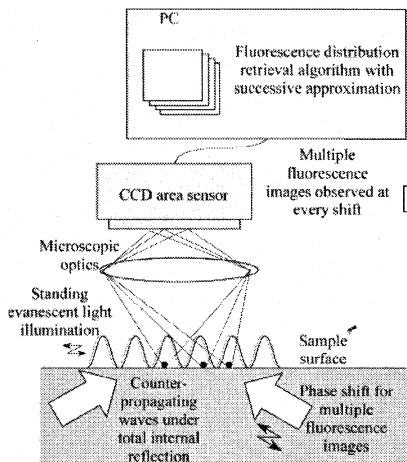


Figure 1: Concept of super-resolution microscopy using standing evanescent light with image retrieval

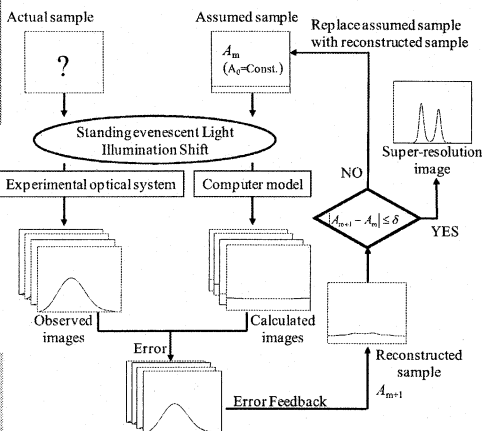


Figure 2: Sample distribution retrieval algorithm from multiple observed images under standing evanescent light illumination

dominated by the diffraction limit. By this method, the multiple images can be detected by shifting the standing evanescent light at a nanometer scale. Since the multiple images and the corresponding illumination distributions are known, we can expect to reconstruct the sample distribution with higher frequency by using successive approximation (Figure 2). Figure 3 shows typical simulation result of the proposed method. In this case, a sample of two discrete dots separated by 227nm, which corresponds to the diffraction limit of the imaging optics ( $N.A.:1.2, \lambda:447\text{nm}$ ) was employed as the fluorescence shift sample on the TIR surface. Figure 3(a) shows the relationship between the position of the two-dot sample and the standing evanescent light illumination ( $T: 98.5\text{nm}$ ). Figure 3(b) indicates the multiple far-field optical images of the two-dot sample with the position of figure 3(a), respectively. Figure 3(c) is a conventional microscopic image obtained under the same condition, and figure 3(d) is a reconstructed super-resolution image calculated from the multiple far-field optical images of figure 3(b).

### 3 Experimental verification of proposed microscopy

Figure 4 shows the microscope system with standing evanescent light illumination shift control unit, which was developed for the verification of the proposed method. This experimental system consists mainly of a CW green laser (Coherent,

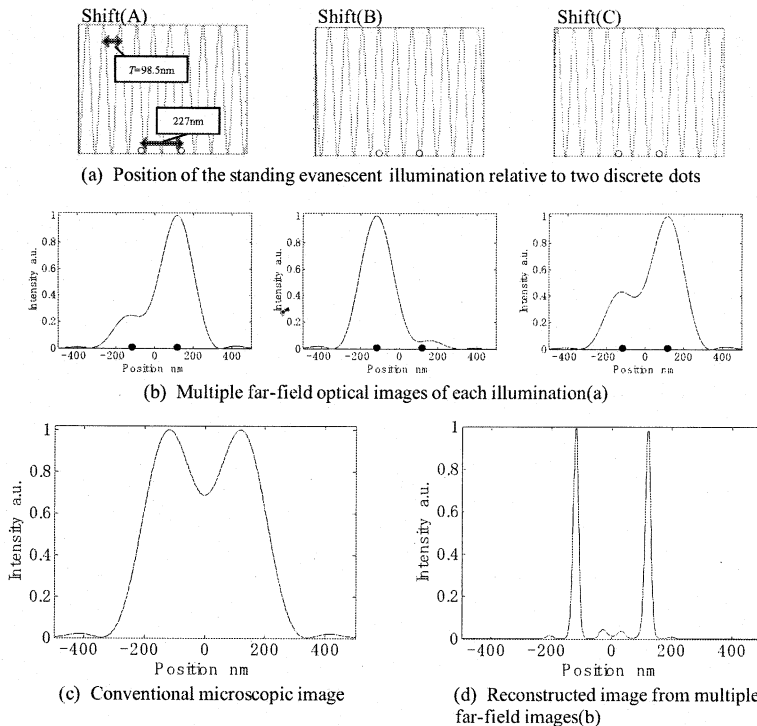


Figure 3: Simulation results of lateral resolution improvement by proposed method Compass315-150) as a linearly polarized light source, a trapeziform prism generating TIR condition on test surface, a piezoelectric translator for shifting the standing evanescent light with the resolution of 1nm, an infinity-corrected optical microscope unit with a 16 bit cooled CCD area sensor (Bitran, BD-40) and a PC, which controls the CCD, the piezoelectric translator, and processes multiple images for super-resolution imaging. As the first step of verification in experiment, we carried out the basic experiment for resolving a discrete sample of standard fluorescent particles with the diameter of 200nm using the lower N.A. objective (0.35). As a result, we can clearly resolve 780nm separated two-dot sample using the optics even with the Rayleigh limit of 927nm, based on our proposed method using standing evanescent light with image retrieval as shown in Figure 5.

#### 4 Conclusions

We verified the proposed lateral resolution improvement mechanism by developing

microscope system based on the active control unit of spatial distribution of standing evanescent illumination. This experimental result suggests that our proposed method enables super-resolution beyond the optical diffraction limit and can be applied to a novel surface inspection method such as nano-defects detection on Si wafer surface, and so on.

**References:**

[1] S. Takahashi, S. Okada, H. Nishioka, S. Usuki, K. Takamasu, Analysis of Lateral Resolution Improvement for Fluorescence Microscopy using Standing Evanescent Light, Measurement Science and Technology, vol.19, 084006 (10pp), 2008

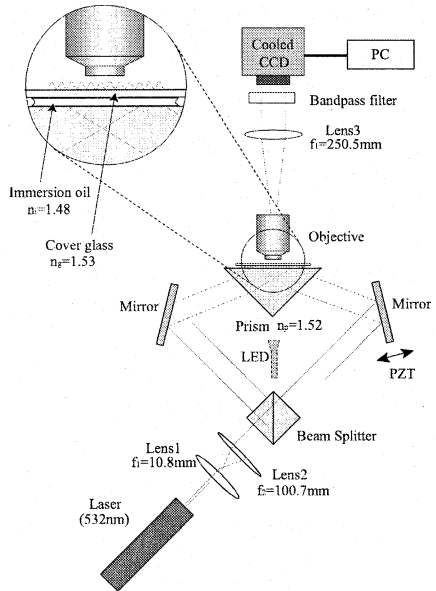


Figure 4: Experimental system for verification of proposed method

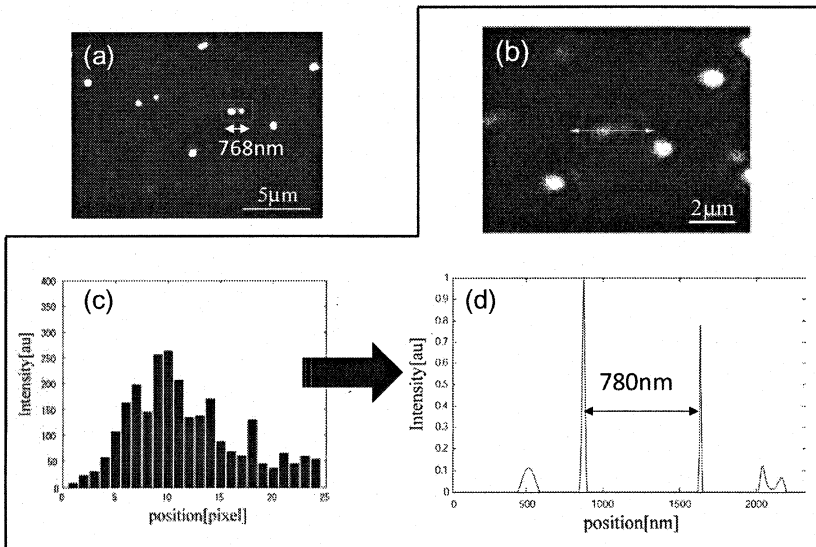


Figure 5: Experimental results obtained with the developed experimental system  
 (a) Standard fluorescent sample (observed with NA0.9: Rayleigh limit: 361nm)  
 (b) Conventional microscope image (observed with NA0.35: Rayleigh limit: 927nm)  
 (c) Intensity profile of image (b) at the cross-section of the arrow position.  
 (d) Super resolution profile (obtained using NA0.35: Rayleigh limit: 927nm)