

Scanning total internal reflection fluorescence microscopy

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ABSTRACT

We report on a new form of total internal reflection fluorescence microscopy. Instead of using a prism, an objective of numerical aperture 1.65 is used under ring beam illumination. As a result, the propagating component of the illumination wave is suppressed and a focused evanescent spot is produced with strength of 20 times stronger than that in the prism. A near-field image is obtained by the scanning of a sample illuminated by the evanescent focal spot. The new imaging system has been successfully used for characterising CdSe quantum dot nanoparticles and will be useful in nano-fabrication and single-molecular detection.

Key works: Near-field microscopy, evanescent wave, optical and innovative microscopy, thin film and surface characterisation.

1. INTRODUCTION

There are two ways to produce total internal reflection microscopy [1]. The first one is a prism-based method and the second one is based on an objective. In both cases, a sample is illuminated by a large area spot and the strength of the evanescent wave is not strong. When a high numerical aperture objective is illuminated by a collimated wave and focused through an interface between a glass cover slip ($n = 1.51$) and a biological sample ($n = 1.33$), an evanescent wave can be produced immediately below the interface due to the total internal reflection caused by the rays of convergence angles larger than the critical angle. The propagating wave produced by the rays of low convergence angles can be suppressed by apodizing the objective. One of the apodization functions is based on the circular obstruction co-axially placed. As a result, a scanning total internal reflection microscope can be constructed for near-field imaging with high transverse resolution and imaging depth less than 100 nm. The strength of the focused evanescent wave can be approximately 20 times stronger than that in the prism method. In this paper, we present theoretical and experimental results of scanning total reflection microscopy using an objective of numerical aperture 1.65. The experimental result is consistent with the theoretical simulation.

2. EXPERIMENTAL SETUP AND RESULTS

The experimental setup of a scanning total internal reflection microscope is shown in Fig. 1. In this new system, the total internal reflection is achieved by introducing a circular obstruction in the optical path of a high numerical-aperture objective (NA=1.65, Olympus). This objective is used with special high refractive indices cover slips and immersion oil, and the sample is scanned over the area of interest. In addition, the utilization of the objective employed increases the illumination angle range beyond the critical angle. The objective lens is characterized using CdSe quantum dot nanoparticles (5 nm in diameter) as a point object.

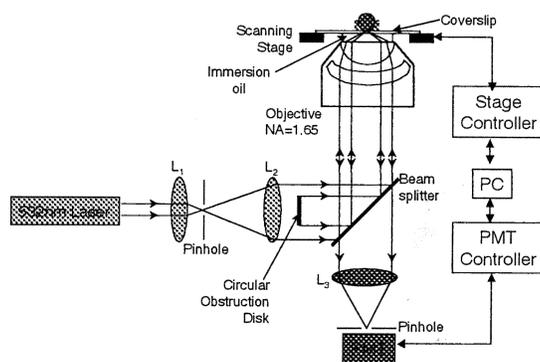


Fig. 1 Experimental setup of scanning total internal reflection microscopy

Fig. 2a shows the image of CdSe quantum dot monolayer in the new system. The concentration of the quantum dots is approximately 1 dot per $3 \mu\text{m}^2$. It is seen that the image of the CdSe dots is slightly elongated along the polarisation direction of illumination. This feature may be caused by the depolarisation in the focal region by a high numerical aperture objective. Fig. 2b is the calculated evanescent focal spot which clearly exhibits the elongation of the spot.

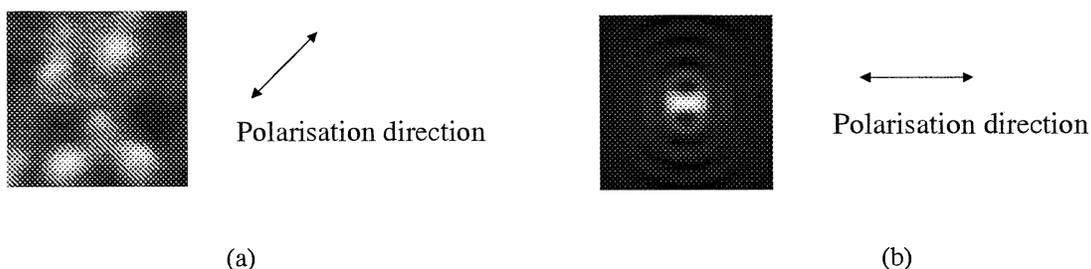


Fig. 2 Experimental image of CdSe dots in the scanning total internal reflection fluorescence microscopy (a). This image shows a slight elongation along the polarisation direction of illumination, which is consistent with the theoretical prediction (b).

3. SUMMARY

Scanning total internal reflection microscopy can be achieved using a high numerical-aperture objective with a central obstruction. As a result, the strength of the focused evanescent spot is enhanced significantly. In fact, if the cover slip is coated with a double layer stack [2], the strength could be increased by four orders of magnitude, compared with that on the prism surface. Such scanning near-field microscopy is useful not only in single-molecule detection but also in nano-fabrication and nano-sensing.

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