

Critical-Angle Illumination for Fluorescence Correlation Spectroscopy with Electron-Multiplying CCD Camera

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Fluorescence correlation spectroscopy (FCS) is a powerful tool for measuring constitutions of molecules in solution by analyzing fluctuation of fluorescence from limited observation volume. By using FCS, we can investigate concentration, diffusion coefficient, molecular dynamics, intermolecular interactions, and so on. In a conventional FCS system, avalanche photo diodes (APD) or photo multiplier tubes (PMT) are used as detectors, because of excellent time responses, good signal to noise ratios and high sensibilities. Since an APD or a PMT is a point detector, simultaneous-multiple point measurement is not possible in the conventional system. Recently, B. Kannan [1] and M. Burkhardt [2] developed a new detection method for FCS with an electron-multiplying charge-coupled device (EM-CCD) camera. Since an EM-CCD camera is regarded as an array of many high-sensitive detectors, the camera can give both FCS data and spatial information simultaneously.

In case of using an EM-CCD as a detector, how to limit the volume size of fluorescence detection becomes a crucial problem. In general sizes of detecting volume in horizontal directions (x and y) are determined by the cell size of EM-CCD detector and the magnification of the microscope, although the vertical direction (z) is not limited. To limit the detecting volume in vertical direction, we introduce a novel illumination method called "critical-angle illumination". In the critical-angle illumination a Gaussian beam is irradiated on a substrate surface at the angle of slightly smaller than the critical angle. In this case the refracted beam travels along the substrate surface and generates electromagnetic field within a few micrometers on the surface (Fig. 1). To investigate the effect of the critical-angle illumination, we have calculated the field intensity distributions in the vicinity of the boundary with a finite difference time domain (FDTD) method. We have also investigated the depth of illumination by the observation with EM-CCD based FCS. Through experiments done with fluorescence particles dispersed in water at a known concentration ($18 \text{ particles}/\mu\text{m}^3$), we have estimated the depth of illumination from the number of fluorescence particles in the observing volumes (Fig. 2). We are aiming to apply this method to live cell observation.

[1] B. Kannan, et al, *Anal. Chem.*, **78**, 3444-3451 (2006)

[2] M. Burkhardt, et al, *Optics Express*, **14**, 5013-5020(2006)

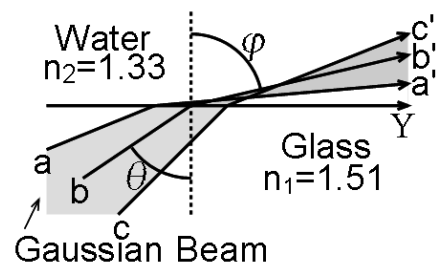


Fig.1 Critical-angle illumination

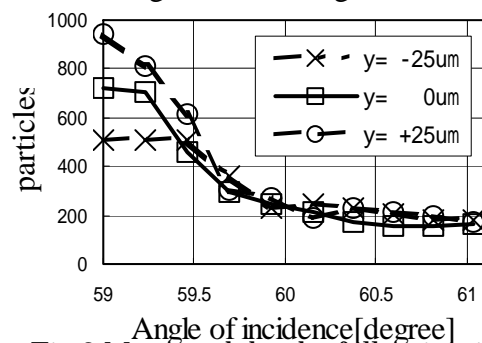


Fig.2 Measured depth of illumination