single-molecular imaging of protein in living cell by pin-fiber video-microscope

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A novel method for single-molecular imaging was developed using pin-fiber video-microscope, which utilizes single optical fiber for sample illumination. This method has several characteristics that include non-use of evanescent light and controllability of illumination area of samples. The light from the optical fiber works as a microscopic spotlight in the dark, and it can illuminate samples with arbitrary area because this illumination system needs no interface for generation of evanescent light.

Single molecular observation of protein inside a living cell was tried by this method. A transcription factor protein (STAT1) was fused to green fluorescent protein (GFP) by gene recombination and introduced into living HeLa cells. The fluorescence from STAT1-GFP was detected by a high-sensitive CCD camera through an image intensifier. STAT1 molecules were recognized as small bright spots when they were recruited to interferon (IFN) gamma receptors and dimerized after IFN gamma stimulation. Under single-molecular imaging condition, movement of STAT1 spots was clearly observed probably due to nuclear translocation of dimerized STAT1 from the cell membrane. By focusing particular STAT1 spot and analyzing time course of the recorded images, it was found that different behavior types of STAT1 image intensity exist. One type was that after appearance of single STAT1 image, image intensity was enhanced to reach a brightness by a factor of 2. The other type was that the STAT1 spot intensity was rapidly increased to this twice brightness. Because the twice brightness might indicate dimerization of STAT1, this fact suggest that slight timing difference of STAT1 dimeriation could be observed by this novel method.