

Single Molecular Fluorescence Obtained Near-field Optical Techniques for Direct DNA Sequence Imaging

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We have visualized hybridized-peptide nucleic acid (PNA) on a stretched DNA strand using scanning near-field optical/atomic force microscopy (SNOM/AFM) in an effort towards investigating the possibility of discriminating continuous DNA sequences on nano scales. A λ -DNA molecule and a self-designed PNA probe were used for the model experiment. A 15-mer PNA probe was designed to hybridize to the top part of the ea47 gene within the λ -DNA molecule. Whole DNA molecules were stained using YOYO-1 and the PNA probe was labeled with an Alexa 532 pigment. Whole DNA molecules were visualized by the fluorescence of YOYO-1 or by topography, and the PNA probe was visualized by the fluorescence of Alexa 532. The resolution achieved of the near-field fluorescence image was 13 nm at the optimum, and topographic images were concomitantly obtained. The technique drastically overcomes the resolution limit of conventional fluorescence in situ hybridization (FISH) techniques achieved by far-field optical microscopy, and enables direct gene mapping at a resolution in the tens of nanometer range. In addition, we will discuss the single molecular discrimination techniques using the SNOM/AFM.