A Novel Approach to Bioimaging via Scanning Near-field Optical/Atomic-force Microscope (SNOAM) Title Title Title

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Introduction

In the bioimaging field there is a growing need for high sensitivity, high spatial resolution, and in vivo monitoring. Electron microscopy (EM) has been powerful tool for giving high-resolution and sensitivity. However it is impossible for EM to perform in vivo monitoring, because of fatal pretreatment of drying, chemical staining or metal coating. Although EM provides better spatial resolution than optical microscopy, EM can not allow observation without preparative procedures, such as fixation, staining and vacuum evaporation. Conventional optical microscopy has also clearly supplied powerful tools for analyses of biological materials. Conventional optics have limit of resolution (approx. 250 nm) which is based on half of wavelength of visible light, while a scanning near-field optical microscopy (SNOM) has demonstrated that spatial resolution of only a few tens nanometers. Its sensitivity, furthermore, up to critical level which can be dome single molecular detection. This technique is analogues to scanning tunneling microscopy (STM) and atomic force microscopy (AFM) as related and derivative method. Various kinds of SNOM have developed with the variation of method to control tip-to-sample separation such as utilizing STM, lateral shear-force and dynamic AFM mode (DFM) since first SNOM was established in 1984. Especially, the appearing of an optical fiber probe accelerated development and modification of SNOM system in 1992. Soon after then, H.Muramatsu and co-workers developed a scanning near-field optical/atomic-force microscope (SNOAM) in which a method of the DFM was used to control the tip-to-sample separation by using a bent-type optical fiber probe. The SNOAM system was first applied to simultaneous topographic and fluorescence imaging of biological materials.

Human Metaphase Chromosome

The structure of human metaphase chromosomes, fixes according to standard procedures for optical microscopy but not treat for banding, was examined by SNOAM observation. The images show that chromosomes, one of the set of bodies in the nucleus which determine hereditarily cell structure and function, display some specific features, detected by SNOAM as a variation in thickness of specific region. This similarity allows the identification of individual chromosomes. The feasibility of an ultramicroscopic system employing a bundle of optical fibers and sensitive optical detection devices for native structure analysis of biomolecule was investigated. Scanning near-field optical/atomic-force microscopy (SNOAM) provided us with simultaneous topographic and near-field optical images of human metaphase chromosomes using a bent-type optical fiber as a cantilever for AFM regulation and a near-field optical probe. The SNOAM system can be performed to obtain nano-scale resolution by atomic-force modulation for sample-to-tip interaction in the air and liquid, respectively. Native chromosomes were spread out onto a coverslip using the "Surface-Spreading Whole-Mount" method. The SNOAM system does not need pretreatment of samples such as metal coating and chemical immobilization. Topographic and near-field fluorescence images revealed useful information on native chromosome structure. Karyotyping is a valuable research tool used to determine the chromosome. The author described that SNOAM system was first applied to simultaneous detection of topographic and near-field fluorescence images of native human metaphase chromosomes which were obtained by cyclic-contact mode. The metaphase chromosomes were derived from human B cell lymphoblastoid line RPMI1788.

GFP-E.coli

Green fluorescent protein (GFP) is a convenient indicator of transformation and should allow cells to be separated by fluorescence-activated cell sorting. GFP was originally isolated from the jellyfish Aequorea victoria and has become a useful reporter molecule for monitoring gene expression and protein localization in vivo and in real time. The gene coding to GFP was cloned in recombinant Escherichia coli. The SNOAM system used 458 or 488 nm irradiation from a multi-line argon ion laser for excitation of GFP, since a native GFP has been known to give a maximum at 395 nm and a broad absorption spectrum until 500 nm. Topographic and fluorescence images of recombinant E.coli were obtained simultaneously with a high spatial resolution which was apparently better than that of a conventional confocal microscope. A nanoscopic GFP fluorescence spectrum was obtained by positioning the optical fiber probe above the bright area of the E.coli cells. Comparing topographic and fluorescence images, it can be seen that individual E.coli cells expressed different fluorescence intensities. Fluorescence obtained by SNOAM indicated that GFP post-translational oxidation possibly occurred near the cell surface. A SNOAM system also indicated the possibility of precise imaging of native cells under the physiological condition, in liquid.