by a focused laser is used as the tension gauge and the marker for the position detection. Single trap geometry, in which single bead trapped by single laser spot, is popular to analyze the behavior of processive motor proteins. With this method, rotational motion of a bead caused with pulling by a biomolecule results in the loss of the motional or structural information when it is deposited around the trap center. To overcome this problem, we have developed the laser trap nanometry with non-spherical shaped probe to control its rotational motion and position. In this study, the duplex bead was used as the probe. To capture this probe, two neighboring laser trapping spots were generated by acoustic optical deflector, and the gap and position of spots are controlled with 20 μ s and 1 nm accuracy. To detect the bead position, the quadrant photodiode system (QPD) with laser dark field illumination method was used. We confirmed the duplex bead is trapped by the neighboring laser trapping spots stably, and act as Hookian spring. QPD detecting system has enough resolution and linearity for detecting the motion of the beads. This method is not only applicable for the analysis of microtubule based non-processive motor for which eyeball method is not work but also for figuring out the intra- and inter-molecule force distribution by controlling the probe position in nanometer accuracy.

3P-314 マイクロアレイを用いた AFM による多数生細胞の力学計測

Force Measurement of living Cells by Atomic Force Microscopy with a Microarray Technique

Yusuke Mizutani (1), Shinichiro Hiratsuka (1), Masahiro Tsuchiya (1), Koichi Kawahara (1), Hiroshi Tokumoto (2) and Takaharu Okajima (1). (1: Division of Bioengineering and Bioinformatics, Graduate School of Information Science and Technology, Hokkaido University; 2: nanotechnology Research Center, Research Institute for Electronic Science, Hokkaido University)

The atomic force microscopy (AFM) is one of the most powerful methods for investigating the mechanical properties of living cells. Since living cells exhibit temporal fluctuations as well as individual differences, especially during the transient states, the statistical information on the mechanical properties of cells is most valuable to elucidate the mechanism of cell mechanics. Therefore, we developed a new method of force measurement by AFM combined with a microarray, which allowed to measure the mechanical properties of a large number of living cells for a short time, so that the number distribution of cells could be estimated.^[1] In this study we demonstrated three experiments of living cells during their transient states by the technique. Firstly, we could obtain the number distribution of fibroblast cells in early stage of adhesion. The results showed that the log-normal distribution was gradually increased as the adhesion time was increased. Secondly, we could obtain the number distribution of cardiac cells from initial state changed by some external stimuli to the final state. The results showed that the log-normal distribution was gradually increased with incubation time after external stimuli. Thirdly, beating cardiomyocytes were cultured in the wells of a microarray. We could measure the mechanical beating of cardiomyocytes. The detailed results and discussion will be presented. This work was supported by grant program from NEDO (06A26007) of Japan. [1] Y. Mizutani et al. Jpn. J. Appl. Phys. In press

3P-315 ATR-FTIR 法によるキンギョのウロコの解析

ATR-FTIR spectroscopic analysis of goldfish scale

Masayuki Nara (1) Atsuhiko Hattori College of Liberal Arts and Sciences, Tokyo Medical & Dental University

A goldfish scale is composed of hydroxyapatite (HAP) and extracellular matrix, mainly type I collagen fibers. We investigated a goldfish scale by infrared spectroscopy to characterize the components of the scale. Attenuated total reflection (ATR)-Fourier transform infrared (FTIR) measurements were performed at 25 °C on a PerkinElmer Spectrum-One FTIR spectrometer equipped with a universal ATR unit and a liquid nitrogen-cooled MCT-detector at resolution 2 cm^{-1} . An ATR measurement has an advantage over a transmission measurement, because ATR technique makes it possible to obtain information about the surface and backside coats separately. Actually, the infrared spectrum for the surface coat of the normal goldfish scale was quite different from that for the backside coat of the same scale. The former showed a strong band at 1013 cm^{-1} , which is due to HAP, and weak bands at 1643, 1415 and 870 cm^{-1} , whereas the latter showed a typical protein profile: strong bands at 1631, 1550 and 1240 cm^{-1} , which are probably assignable to amide-I, amide-II and amide-III of collagen, respectively. We also examined allotransplanted goldfish scales by ATR-FTIR spectroscopy. After two weeks transplantation, the intensity of the band due to HAP for the allotransplanted goldfish scale was weaker than that for the normal one. This result suggests that the HAP layer of the allotransplanted scales is partly destroyed by osteoclasts. ATR-FTIR spectroscopy is promising for monitoring HAP and collagen components on the process of osteogenesis of goldfish scale.

3P-316 一次イオン源 C60 と Ga を用いた飛行時間型二次イオン質量分析法によるタンパク質表面構造測定の比較検討

Comparison of primary ion sources, C60 and Ga, on protein measurement with time-of-flight secondary ion mass spectrometry

Fuyuko Takaki, Satoka Aoyagi Faculty of Life and Environmental Science, Shimane University

Primary ion source effect on spectra of protein samples was evaluated using time-of-flight secondary ion mass spectrometry (TOF-SIMS). Though TOF-SIMS is expected to very useful to evaluate proteins on bio-devices, it has not been widely employed in bio fields yet because secondary ion generation phenomena of TOF-SIMS measurement for bio-molecules such as protein have not been

clarified. In this study, two types of primary ion sources were compared to evaluate fragmentation of protein molecules and depth of secondary ion generation. Streptavidine was employed as a model protein. Streptavidin and quantum dot-labeled streptavidine were immobilized on aminosilanized indium tin oxide (ITO) coated glass plates at epsilon-amino group in lysine of the protein. The samples were freeze-dried before they were measured with TOF-SIMS. Each sample was measured with a monoatomic primary ion source, Ga⁺, and a cluster ion source, C60, respectively, and spectra from both primary ion sources were compared. As a result, differences of fragment ions from the same protein depending on primary ion sources were found, which indicates differences of secondary ion generation depth. High mass secondary ions were generated with the cluster ion source and secondary ions including substrate or quantum dot related materials were obtained with the monoatomic ion source. Therefore it is indicated that the selection of appropriate primary ion source is very important to obtain information on immobilized protein molecules depending on analysis purposes.

3P-317 走査型電子顕微鏡観察用金属ラベル：元素別識別方法

A universal method for making metal nanoparticle labels for scanning electron microscopy: tagging based on the element species

Hiroyuki Takei (1,2), Hyonchol Kim (1), Kenji Yasuda (1,2). (1: Kanagawa Academy of Science & Technology; 2: Dept Biomedical Information, Div Biosystems, Inst Biomaterials & Bioengineering, Tokyo Medical & Dental Univ)

Scanning electron microscopy (SEM) is a powerful tool for extracting highly detailed structural information from biological specimens. It is particularly true when a cell can be stained with gold colloids modified with a specific antibody or DNA fragment that can target particular biomolecules within the cell. Its potential has not been fully exploited, however, because multiplexing, targeting of multiple biomolecules simultaneously, has not been carried out due to lack of labels that can be tagged for identification of different biomolecules. Our concept for overcoming this issue is based on a fact that metals with different atomic numbers scatter electrons differently when viewed in the backscattered mode. We make use of nanoparticles with different metals, coated with various biomolecules. They are prepared by coating of Latex nanospheres with metals with thermal evaporation; a monolayer of Latex spheres is formed on a substrate, then a metal(s) can be evaporated, and after attachment of a probe biomolecule onto the metal coated Latex sphere, spheres can be removed under mild application of ultrasound. We will show that such metal nanoparticles can be readily identified on the basis of the metal element, the thickness of coating, the size of the Latex sphere and the composition of the metals. These are ideally suited for tagging a large number of biomolecule simultaneously, possibly up to 100 species and beyond, and we believe that a new research area will open up with multiplexed labels for SEM observations.

3P-318 ビオチン-アビジン相互作用の分子認識力画像化

Molecule recognition Imaging of biotin-avidin interaction

Masato Kawano, Hitomi Hokonohara, Toshio Kawahara Takuya Matsumoto and Tomoji Kawai (ISIR, Osaka Univ.)

The formation and dissociation of specific non-covalent interaction between various of macromolecules such as protein which plays a crucial role in the function of biological systems. When we know microscopic properties of the interaction between protein molecules, treating biological disorders, structure-based drug design, purifying protein mixtures and protein diffusions should have much progress. Atomic force microscopy (AFM) has been a promising method to elucidate bio-molecule interaction with single-molecule level resolution. AFM is a powerful tool and widely used for the imaging of bio-molecules, where recently force spectroscopy has also been used for the detection of dynamical properties such as a binding force of the molecules. However, in usual, molecular interaction is analyzed with the use of force-curve measurement with a lack of spatial resolution. In this paper, we propose a method for imaging attractive interaction between biological molecules concurrently with taking topography in water. For this purpose, the frequency-shift variation is detected for attractive force imaging and the amplitude is kept constant by feedback operation to obtain topography. In comparison with an envelope analysis method reported recently, our approach is expected to have advantages in sensitivity, response, and less cross talk between topography and recognition image. Furthermore, we examined the effect of amplitude of cantilever oscillation on topography and attractive force image indicating the distortion properties of proteins.

3P-319 カーボンナノチューブのカンチレバー探針への適用

Direct growth of CNT at the cantilever tip

Kensuke Suzuki(1), Masahiro Imai(1), Masaki Tanemura(4), Yoshitaka Sugita(5) and Toshio Andou(2)(3)(1: Department of Mathematics and Physics, Grad School of Natural Science and Technology, Kanazawa Univ; 2: School of Mathematics and Physics, College of Science and Engineering, Kanazawa Univ; 3: CREST/JST; 4: Nagoya Institute of Technology, Department of Frontier Materials; 5: Nagoya, Department of social engineering)

Biological macromolecules are highly dynamic. Their functions result from their dynamic structural changes and dynamic interactions with other molecules. Therefore, directly visualizing their dynamic behaviors will tell us, in a straightforward manner, how their functions are produced. We have been developing high-speed atomic force microscopes to materialize this type of visualization. We can now capture moving protein molecules on video at 30-60 ms/frame, without disturbing their physiological functions significantly. However,