can see how the leg-like anchor domains of FliD bind to FliG, in a similar manner to the structure of the D6-D1 domain of Flc over the symmetry mismatch as well as the depression and space inside the cap complex for promoting assembly.

3PS13 パイメタルカンチレバーを用いた褐色細胞微細の発熱量の定量的解析
Quantitative analysis of heat production of brown adipocytes using bimetal cantilever
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Brown adipocyte is a cell specified for metabolic heat production. From a calorimetry using 10³ numbers of the cells, the tissue is thought to produce nW-ordered heat. However, the character of each cell cannot be investigated by the calorimetry. To reveal the character, the measurement technique for heat production of a single cell is needed. So we developed a bimetal cantilever (750 × 40 μm), which is composed of gold (100 nm in thickness) and silicon nitride (200 nm in thickness). When the cantilever senses heat, it bends due to the difference of the coefficient of thermal expansion of the metals. We tried to measure the heat produced by a small number of the cells using the cantilever. The displacement of cantilever was measured under a conventional microscope when the 5-6 cells reached about 2 μm away from the tip of cantilever. After the addition of norepinephrine (final 1 μM), the displacement of the cantilever increased as time passed. After 60 min from the addition, the displacement of cantilever became maximum value (60 nm). If the heat which the cantilever detected has escaped only at the end of the cantilever, the value corresponds to that the temperature of the tip of the cantilever has increased 10 mK. After further 90 min, the displacement was not observed. We would like to discuss the activation and inactivation of heat production of the cell together with lipid consumption of the cell and expression level of protein which is involved in heat production.

3PS041 郷ional Conference 2012 (2012) on the future of science education in the fields of biophysics, nanotechnology, and biology
Time-dependent Poisson’s ratio and Power-law rheology of cell sheet in uniaxial stretching experiment
Masahiro Tsuchiya, Yusuke Mizutani, Takaharu Okajima (Graduate School of Information Science and Technology, Hokkaido Univ.)

Mechanical properties of living cells are strongly related to their physiological functions such as migration, division and restructuring of their intracellular structures. Rheological properties of living cells have been extensively investigated by various microbead techniques, atomic force microscopy and single cell stretching method. These studies showed the single cell exhibited a power-law behavior in frequency domains. The Poisson’s ratio of living cells is of the important modus and related with some time dependent modulus. Moreover, studies regarding polymer materials revealed that the Poisson’s ratio was a function of time. However, the time-dependent Poisson’s ratio of cells has not been measured. In this study, we measured the Poisson’s ratio of cells as a function of time during stress relaxation of cell sheet composed of NIH3T3 cells with a size of ca.500 × 400 μm. We found that the Poisson’s ratio of cell increased with time and became time constant. As the actin filaments were disrupted, the time dependent Poisson’s ratio reduction is rapidly changed. The results indicated that the time dependent Poisson’s ratio was strongly related to the contraction of actin structures.

3PS05 原子間顕微鏡を用いた単一細胞ロジジーの高速・精密測定法の開発
High-speed measurement of single cell rheology with an atomic force microscope
Ryoosuke Takahashi, Satoshi Ichikawa, Takaharu Okajima (Graduate School of Information Science and Technology, Hokkaido University)

Single cell measurements of cell mechanics are crucial not only for understanding various cell functions such as migration and proliferation but also for distinguishing different types of cells. It has been reported that the elastic property of cancer cells is smaller than that of normal cells [1, 2]. We obviously observed that cancer cells are softer and more fluid than normal cells and found the difference of the ensemble distribution of rheological properties between normal and cancer cells such as human mammary epithelial cells (MCF-10A) as normal cell and human mammary adenocarcinoma cells (MCF-7) as cancer cell [7]. To improve throughput of cell measurements, we developed an AFM system for the measurement of rheological properties of a large number cells, the shape of which was controlled with micropatterned substrates [3], in the frequency domain [4, 5]. The observed AFM data could be analyzed using a power-law structural damping model [6]. We will present the detailed AFM system and discuss the ensemble distribution of power-law rheology parameters between normal and cancer cells.


3PS06 “ホッピングモード” 高速原子間力顕微鏡(HS-AFM)の開発
Development of “hopping-mode” high speed atomic force microscopy (AFM)
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High-speed AFM (HS-AFM) can directly visualize dynamic behaviors of single protein molecules in action and has recently been proven to be powerful in the elucidation of functional mechanisms of proteins [1-3]. However, it is hard to observe large and soft biological samples such as live cells and organelles using our tapping-mode HS-AFM because the AFM cantilever tip exerts relatively large lateral forces to the sample during scanning and thus damages soft samples.

To overcome this problem, we attempt to combine a new operation mode of AFM called hopping mode [4] with the HS-AFM. In hopping-mode AFM, we no longer use continuous feedback operation. Instead, at each imaging point, the AFM tip approaches the sample from a starting position that is above any of the surface features. The cantilever is freely oscillating when the tip is well away from the surface. The tip then approaches until the amplitude is reduced to a predefined value. When the amplitude experiences this reduction, the position of the z-piezo is recorded as the sample height at this imaging point. Owing to this operation principle, the tip lateral forces applied to the sample can be significantly reduced even for tall samples. In this presentation, we will show the capability of this new AFM system by presenting captured images of HeLa cells.


3PS07 経済的高次超電磁界の開発に向けた基礎研究
Pilot study for the development of high-speed ultrasonic AFM

We have developed high-speed AFM (HS-AFM) that enables us to directly visualize dynamic structural changes of protein molecules at high spatiotemporal resolution. So far, various biological processes performed by protein molecules have been directly filmed as molecular movies, which allowed us to gain insights into their functional mechanisms much more straightforwardly than other techniques [T. Ando, Nanotechnol. (2012)]. However, the observations by HS-AFM are generally limited only to phenomena occurring on relatively hard surfaces, meaning that we can observe neither objects placed on a soft surface (e.g., protein molecules on mammalian cell surfaces) nor objects placed under a surface (e.g., organelles and cytoskeletons in the interior of cells). Ultrasonic techniques must be useful in breaking this limitation because they have been widely used for non-invasively imaging of objects lying on/under a surface, whereas their spatial resolution is limited by the wavelength of ultrasonic used. However, AFM which is combined with ultrasonic techniques is recently claimed to be able to perform subsurface imaging of objects in the interior of cells at nanometer resolution [GS. Shekhawat & VP. Dravid, Science (2005)]. Thus, we are currently attempting to find a way to combine our HS-AFM with ultrasonic techniques. In the presentation, we will report our pilot study on this subject.

3PS08 原子間顕微鏡を用いたタンパク質製造系の研究
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