【Grant-in-Aid for Scientific Research(S)】

Integrated Science and Innovative Science (New multidisciplinary fields)

Title of Project : Study of molecular interaction of molecular complexes in live cell using multipoint temporal and spatial correlation spectroscopy analysis.

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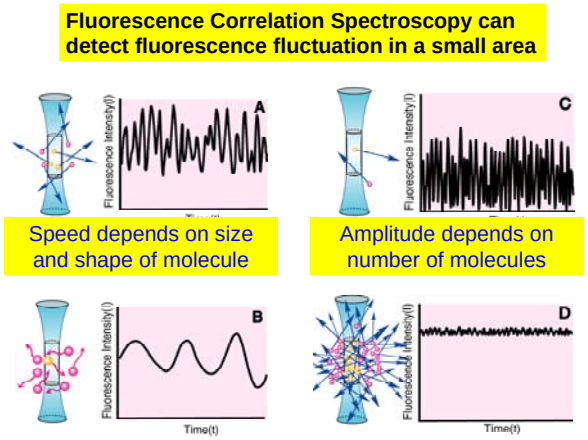
Research Area : New multidisciplinary fields

Keyword : Single-molecule science

【Purpose and Background of the Research】

FCS (fluorescence correlation spectroscopy) detects fluorescence intensity fluctuations caused by Brownian motion of fluorescent tagged molecules in a tiny detection volume (~ femtoliters) generated by confocal illumination. Through time correlation analysis of the fluorescence fluctuations, the diffusion coefficient, molecular concentration and molecular interactions of probe molecules are accessible (Fig). Since FCS needs only a very small detection volume and has high sensitivity, it is also useful to measure diffusional mobility of proteins in very small regions of subcellular microenvironments in living cells.



In spite of their wide range of applications, FCS measurements are restricted to monitoring at only one volume element defined by both a focused laser beam and a pinhole.
To overcome this restriction, we developed a multipoint FCS system which was based on an objective-type total internal reflection-FCS (TIR-FCS). We simultaneously determined diffusion coefficients at different seven points of a cell membrane and shown heterogeneous structure of the cell membrane using this system. However, the area of the measurement is restricted to only the surface, that is, the plasma membrane. Therefore, the purpose of this project is further development of a multipoint FCS system that has three-dimensional capacities and can analyze the molecular complexes and molecular interactions at any point in a living cell.

【Research Methods】

The new multipoint FCS will be constructed using a liquid-crystal-on-silicon (LCOS) spatial light modulator (SLM) that can produce multiple laser beams by a holographic pattern and use a multichannel detector system. The sensitivity of the system will be enhanced by cross correlation measurement using two spectrally distinguishable fluorophores. The feasibility of new system will be verified through an observation of biomolecular interaction such as transcriptional factors in living cell.

【Expected Research Achievements and Scientific Significance】

Recently, advanced microscopic methods provide powerful and important tools for the analysis of protein dynamics within cells by fine imaging of complex structures. Therefore, this research project will create a new system for imaging of biomolecular dynamics and interaction in living cells, and the development of a new field of single-molecule bioinformation science is thus anticipated. Besides its application to single cells, this research is expected to help clarify cell-cell communication through its application to multicellular systems.

【Publications Relevant to the Project】

Yu Ohsugi, Kenta Saito, Mamoru Tamura and Masataka Kinjo.
Lateral mobility of membrane-binding proteins in living cells measured by total internal reflection fluorescence correlation spectroscopy.
Biophys. J. 91, 3456–3464 (2006)

Yu Ohsugi and Masataka Kinjo:
Multipoint fluorescence correlation spectroscopy with total internal reflection fluorescence microscope. *J. Biomedical Optics* 14(1), 0140301-0140304, (2008)

【Term of Project】 FY2009-2013

【Budget Allocation】 135,900 Thousand Yen

【 Homepage Address and Other Contact Information】

http://www.lfsci.hokudai.ac.jp/labs/infmcd/index.html