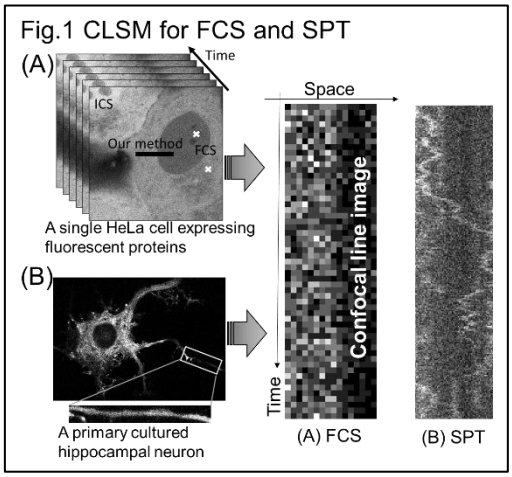
Confocal laser scanning microscopy for FCS and SPT

Kazunari Mouri[1], Yasushi Okada[1,2]
[1]Center for Biosystems Dynamics Research (BDR)
RIKEN
Osaka, Japan
[2]Department of Physics, Universal Biology Institute (UBI) and
International Research Center for Neurointelligence (WPI-IRCN)
The University of Tokyo,
Tokyo, Japan

Fluorescence (cross) correlation spectroscopy (FCS/FCCS) is an established single-molecule based method for a variety of measurements in the field of protein science, such as estimating the concentration and diffusion coefficient of proteins, which enables to calculate a dissociation constant (Kd) of proteins, and so on. However, it needs an expensive dedicated set-up with appropriate choice of fluorescent proteins (FPs) or chemicals. Furthermore, we have to correct a leak of emission signals especially in FCCS methods. Thus its application in cell biology is still limited and a challenging work. To overcome these problems, we developed a new FCS/FCCS methods based on the confocal laser scanning microscopy (CLSM) equipped with high speed scanner combined with wavelet analyses (Fig.1A). CLSM is usually applied in image correlation spectroscopy (ICS), but it's only for molecules on plasma membrane or the accuracy is limited for soluble proteins. Our methods succeeded in accurate and multi-point calculation of auto- and cross-correlation functions in living cells [1]. In addition, the cross correlation between pixels enabled us to extract directional "flow" of proteins which have only been achieved by FCS equipped with multi-detector systems. Careful selection of scan conditions, objective lens, and fluorescent chemicals, enabled us to capture single motor proteins moving along an axon of mouse hippocampal neurons. A single particle tracking (SPT) can be applied for a measured image sequence (Fig.1B).



[1] Y. Fujioka, J. M. Alam, D. Noshiro, K. Mouri, T. Ando, Y. Okada, A.I. May, R.L. Knorr, K. Suzuki, Y, Ohsumi, and N.N. Noda, "Phase separation organizes the site of autophagosome formation", *Nature*, in press.