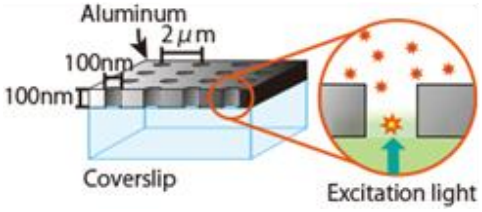
Functional Analysis of Biomolecules using Single-molecule Imaging Technique

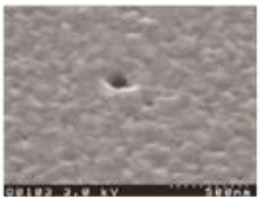
Kyoto University [1], ○Yoshie Harada[1]

E-mail: harada.yoshie.4r@kyoto-u.ac.jp

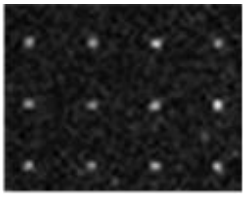
The best way to obtain unambiguous information about the function of biomolecules is to study their function at the single-molecule level. Imaging of individual molecules under an optical microscope is useful for understanding the working principle of biomolecular machines. We developed a single-molecule fluorescence imaging technique using total internal reflection fluorescence microscope in 1995. We can observe single fluorescence labeled biomolecules now. However there are some limitations and problems. For a high background noise, it is very difficult to visualize single fluorescence labeled biomolecules in the presence of fluorescence molecules more than 50nM. Photobleaching and blinking disturb imaging of molecules for a long time. In addition, it is difficult to select probe signal from auto fluorescence in cell and *in vivo* measurements. Therefore we are developing new single-molecule fluorescence imaging techniques. One is zero-mode waveguides (ZMWs) and the other is a new technique using nanodiamonds. I will show you those techniques and examples of functional analysis of biomolecules using those techniques.



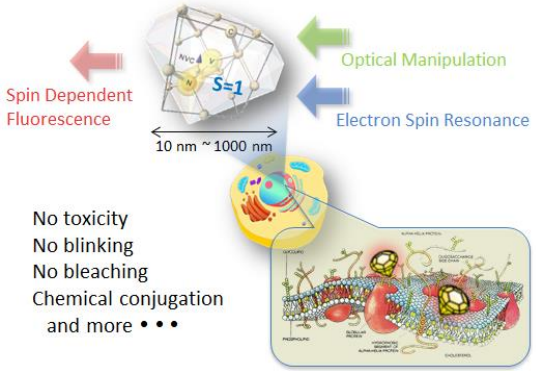
Schema of zero-mode waveguides (ZMWs)

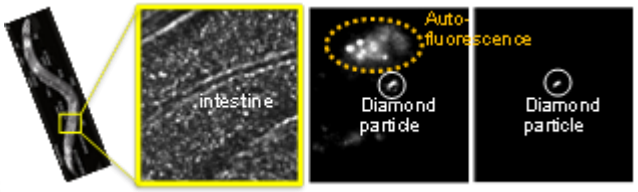


STM image of ZMWs



Fluorescence image of fluorescent dye in ZMWs





Selective observation of diamond nanoparticles in *C. elegans*

1. T. Iwasa, Y.-W. Han, R. Hiramatsu, H. Yokota, K. Nakao, R. Yokokawa, T. Ono & Y. Harada "Synergistic effect of ATP for RuvA–RuvB–Holliday junction DNA complex formation" Scientific Reports (2015) **5**, 18177.
2. R. Igarashi, Y. Yoshinari, H. Yokota, T. Sugi, F. Sugihara, K. Ikeda, H. Sumiya, S. Tsuji, I. Mori, H. Tochio, Y. Harada & M. Shirakawa "Real-time background-free selective imaging of fluorescent nanodiamonds *in vivo*" Nano Letters (2012) **12**, 5726–5732.