IRIS super-resolution microscopy enables to image multiple protein distributions in a single cell

Tai Kiuchi1, Naoki Watanabe1,2

1Medicine, Kyoto University, Japan, 2Biostudies, Kyoto University, Japan

Cellular phenomena such as cell division and migration are regulated by various proteins. To study the complex cellular systems, super-resolution microscopy that can visualize protein distribution with a resolution of 20-100 nm is useful. But the conventional super-resolution microscopy has a limitation in the number of observable targets due to the number of usable fluorescent dyes (generally two or three). Also the super-resolution microscopy reveals the uneven labeling pattern of antibodies and fluorescent proteins on the target distribution with the high resolution. This means that imaging of the accurate distribution of targets is very difficult with the conventional labeling method. To solve the two problems, we recently have developed a multitarget super-resolution microscopy called image reconstruction by integrating exchangeable single-molecule localization (IRIS) (Kiuchi et al., Nature Methods, 12: 743-746, 2015). In IRIS, exchangeable probes that repeatedly associate with and dissociate from their targets are used for labeling, instead of antibodies and fluorescent proteins. The localization of the binding probe is determined as the central position of the fluorescent speckle with high accuracy. Integration of the positions produces the high resolution image of the target distribution. Washing out the probes and adding new probes one after another allow for the sequential imaging of multiple targets within a single specimen. In addition, the repeated associations of the probes enable to increase the labeling efficiency in the target distribution unlimitedly. To verify the IRIS method, we imaged actin filaments using Lifeact that repeatedly associates with and dissociates from actin filaments. The super-resolved image demonstrated that the labeling density of Lifeact readily reached 60 times the maximum labeling density attainable with antibody binding. For multitarget IRIS imaging, we screened 18 IRIS exchangeable probes out of 46 fragments of 15 cytoskeleton- or focal adhesion-associated proteins. IRIS using these probes discerned the area-specific proximity of actin filaments, microtubules, intermediate filaments and focal adhesions within a single cell (Fig. 1). Thus IRIS approach overcomes the limitation on labeling efficiency and enables to image the multiple target distributions with high fidelity.