Meso/Nano Correlative Imaging by Multiplex Protein Meso-Imaging with SIMS and Single Protein Nano-Imaging with HIM

Hee Jin Lim[1], YoungHo Park[2], SunYoung Lee[1], Suil In[2], DaeWon Moon[1]

[1.] Department of New Biology, DGIST, Daegu, Korea
[2.] Department of Energy Systems, DGIST, Daegu, Korea

Recent development in mass spectrometric (MS) imaging for biological specimen has been quite impressive such as matrix assisted laser desorption and ionization (MALDI), secondary ion mass spectrometry (SIMS), and DESI (desorption electrospray ionization). We have developed ambient MS imaging called nano-PALDI (particle assisted laser desorption and ionization) demonstrating molecular imaging of live mouse hippocampal tissues with sub-cellular resolution. [1] Among MS imaging mentioned, SIMS has the highest spatial resolution for imaging but SIMS imaging is limited to lipids imaging. We are trying to develop a multiplex protein SIMS imaging method by antibody conjugated metal oxide nanoparticle(MONP)s utilizing the extremely high secondary ion yield of MONPs for SIMS ion yield amplification and the availability of several tens of different MONPs over the limit of confocal fluorescence imaging up to 3 proteins. Meso-scale MS imaging can be extended to nano-scale imaging of single proteins conjugated with a MONP with He Ion Microscopy (HIM) of 0.5 nm spatial resolution.

A SIMS imaging of AMPA receptor in a mouse hippocampal CA1 region is shown in Fig. 1, where AMPA receptor proteins are stained by Fe3O4 MONP conjugated with AMPA receptor antibodies. SIMS imaging of AMPA shows the distribution of AMPA receptors in the CA1 region of a mouse hippocampus indicating high concentration of AMPA receptors in the soma region and low concentration in the apical dendrite region. The same regions were imaged by HIM with 0.5 nm spatial depth resolution. HIM imaging clearly shows clearly the different distribution of AMPA receptors in the two region as in Fig. 1.

Since tens of different MONPs can be used for conjugation of various antibodies, the number of proteins to be imaged by SIMS can be extended far beyond the limit of confocal fluorescence imaging. In addition to the multiplex protein imaging in the meso-scale, HIM can provide nano-scale imaging in the single protein level, which may generate totally different and innovative histological imaging platform technology for cancer researches, neuroscience, and cardiovascular disease researches.

HIM does not have a potential for elemental identification, since the imaging is based on secondary electron emission like electron microscopy. However, by using MONPs with different size or different secondary electron yield, a couple of proteins may be identified by the different size or brightness of HIM images of MONPs. The possibility of identifying a couple of different proteins by HIM may be demonstrated.

Imaging of multiplex proteins by SIMS suggests the possibility of assessing co-localization or proximity of proteins in a tissue, which should be clear biological implications for understanding disease mechanisms. Multiplex protein imaging by SIMS will be assisted by complementary HIM imaging of a couple of proteins in the nano-scale. We propose that correlative imaging of meso/nano correlative imaging by multiplex protein meso-imaging with SIMS and single protein nano-imaging with HIM will be a useful and innovative imaging platform for various bio and medical areas.

References:

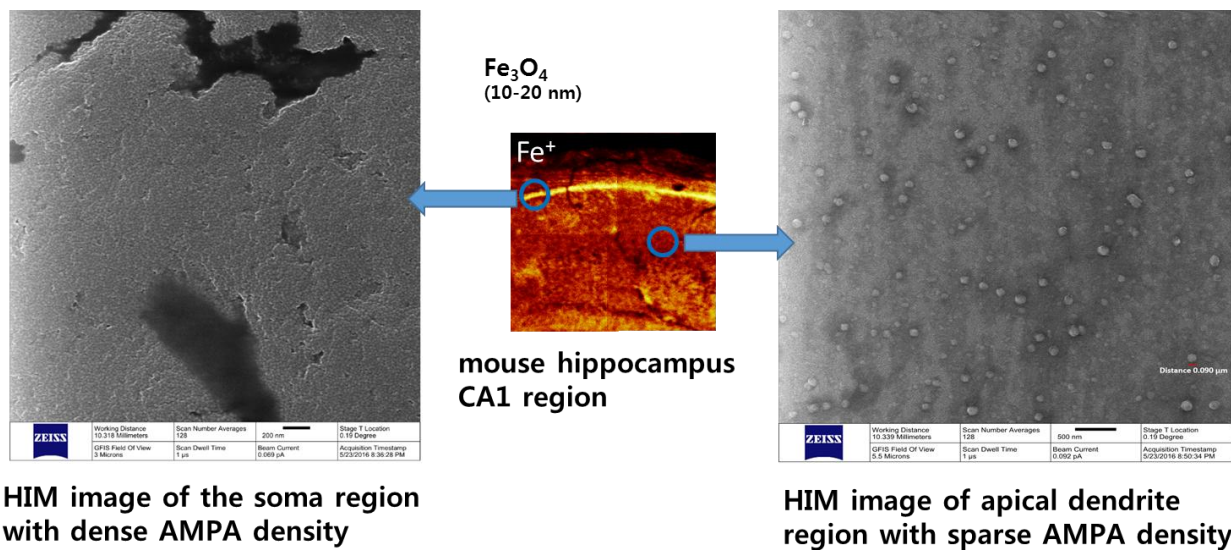[1] Y. Kim, E. S. Seo, H. Kim, J.-W. Park, D.-K. Lim and D. W. Moon, Nat. Commun. 8 (2017), 2113.

Figure 1. Correlation of SIMS imaging (center) of AMPA receptor in the CA1 region of a mouse hippocampal tissue and HIM imaging demonstrating the soma region with dense AMPA receptor density (left) and the apical dendrite region with sparse AMPA receptor density (right).