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

Biological Sciences (Agricultural sciences)

Title of Project：Direct visualization of the in vivo distribution of chemical components in plant cells by TOF-SIMS

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Research Area：Biosciences・Agriculture・Forestry・Wood Science

Keyword：tissue structure, lignin, extractives, conservation and culture of woods

【Purpose and Background of the Research】

Time-of-flight secondary ion mass spectrometry (TOF-SIMS) is a surface-sensitive mass spectrometric technique with high mass resolution and provides chemical information about the surface of a solid sample without any chemical pretreatments. A significant advantage of TOF-SIMS over other techniques is its ability to obtain mass spectral imaging, which allows the direct location of the molecular or fragment ions of interest at a cellular level with submicrometer spatial resolution in biological samples. In this work, we apply TOF-SIMS technique to directly visualize chemical substances in flash frozen plant tissues. This will allow the in vivo location of intra and extra-cellular components in biological samples.

【Research Methods】

We are planning to build a cryogenic system providing direct determination of frozen samples by improving a TOF-SIMS TRIFT III instrument in Nagoya University (Figure 1). First, a load-lock chamber allowing flash frozen samples to insert and rapidly transfer to a TOF-SIMS measurement stage is necessary. The sample stage and the analysis stage are connected under controlled temperature and pressure. It is important to maintain cellular integrity of biological samples in TOF-SIMS analysis.

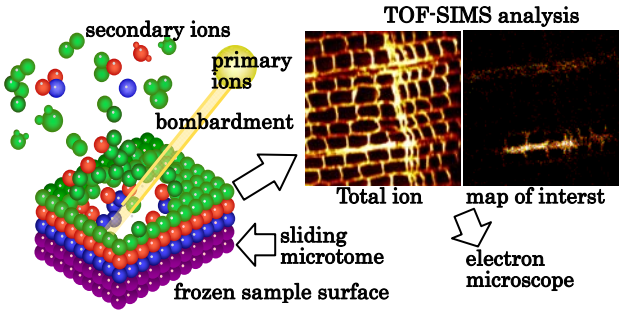

Fig.1. Schematic diagram of a cryogenic system for TOF-SIMS analysis.

Second, we establish optimum conditions for frozen sample measurement using molecular ions of chemical components in plant cell walls, such as lignin, polysaccharides, and extractives. The molecular ions of cell walls constituents have been identified previously. Finally, we also improve other parts necessary for cryogenic TOF-SIMS analysis, which include primary ion beams, an electron microscope, and a sliding microtome.

【Expected Research Achievements and Scientific Significance】

There are few studies that apply TOF-SIMS molecular imaging for frozen plant tissues. It is difficult to detect molecular ions from frozen samples under high vacuum with high spatial resolution. The molecular mapping in the cryogenic TOF-SIMS system in this project is expected to directly analyze the distribution of chemical constituents in plant cells including the vacuoles, such as water soluble components and storage materials.

【Publications Relevant to the Project】

・K. Saito, T. Mitsutani, T. Imai, Y. Matsushita, K. Fukushima, Discriminating the indistinguishable sapwood from heartwood in discoloured ancient wood by direct molecular mapping of specific extractives using ToF-SIMS, *Anal. Chem.*, **80**, 1552-1557 (2008)

・K. Saito, K. Fukushima, Application of TOF-SIMS in the chemistry of plant cell walls, *Regulation of plant growth and development*, **43**, 156-163 (2008) (in Japanese)

・K. Saito, T. Kato, Y. Tsuji, K. Fukushima, Identifying the characteristic secondary ions of lignin polymer using ToF-SIMS, *Biomacromolecules*, **6**, 678-683 (2005)

【Term of Project】 FY2009- 2013

【Budget Allocation】 85,100 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.agr.nagoya-u.ac.jp/~lignin/