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Review

More than Pictures: When MS Imaging Meets Histology

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Attaining high-resolution spatial information is a recurrent challenge in biological research, particularly in the case of small-molecule distribution. Mass spectrometry imaging (MSI) is an innovative molecular histology technique that could provide such information. It allows *in situ* and label-free measurement of both the abundance and distribution of a variety of molecules at the tissue or single cell level. The application of MSI in plant research has received considerable attention; thus, in this review, we describe the current state of MSI in plants. In particular, we present an overview of MSI approaches, highlight the recent technical and methodological developments, and discuss a range of applications contributing to the field of plant science.

Trends

Understanding the chemical complexity of plants requires novel technologies to track metabolites at the highest spatial resolution.

MSI is emerging as an advanced and essential molecular histology approach to map the distribution of plant molecules.

Recent technical and methodological advances in MSI technology have permitted its application in various fields of plant research.

Spatial Localization: The Missing Dimension in MS-Based Omics

The 'omics revolution', particularly for proteomics and metabolomics, is closely associated with technological development in MS. MS is currently the most efficient technology for molecule structural characterization, which has made significant inroads into providing a comprehensive understanding of biological functions [1]. Unfortunately, spatial information is frequently lost in MS-based holistic studies in which the analysis is performed on a tissue homogenate [2]. Significantly, higher plants comprise ten basic tissue types with approximately 15 structurally diverse cell types, which makes spatial analysis imperative [3]. The proteome and metabolome products measured within plants are all dynamic and spatial resolved [4]; therefore, knowing the spatial organization of the molecules at both the tissue and cellular levels will provide fundamental insights into plant biology [5].

A few techniques have been coupled off-line with MS to recover missing spatial information [6]. These techniques are typically based on the *in vitro* isolation and extraction of individual tissue and/or cell types, in which **fluorescence-activated cell sorting** (FACS) (see Glossary) and **laser-capture microdissection** (LCM) are the most prevalent tools [7]. Using such methodologies is operative in obtaining unique spatial information. However, they are typically time consuming and the molecules may undergo modification or degradation during sample preparation [8,9].

Mass Spectrometry Imaging in a Nutshell

MSI is an MS-based molecular histology technique that inherits from MS the unique advantages of high sensitivity, wide dynamic range, excellent molecular specificity, reasonable semiquantification capability, and the versatility to address many varied molecules in a single analysis [9]. The most distinctive advantages of MSI over other imaging techniques are its wide chemical identification capability and no requirement for detailed prior knowledge of the sample composition [10].

The basis of MSI is the **mass spectrometer**, which has three major parts: ion source, mass analyzer, and detector. In a prototypical MS experiment, the sample is delivered into the mass spectrometer, ionized, and vaporized in the ion source, and the resultant ions are sorted according to their mass:charge ratio (*m/z*) in the mass analyzer. Ions are finally detected in

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the detector, and a plot of ion abundance against *m/z* represents a 'mass spectrum' [11]. Two acquisition modes are used in MSI, microprobe and microscope (Figure 1), which differ significantly in how the spatial information is obtained [12]. In the microprobe mode, a focused laser (e.g., matrix-assisted laser desorption ionization; MALDI) or primary ion beam (secondary

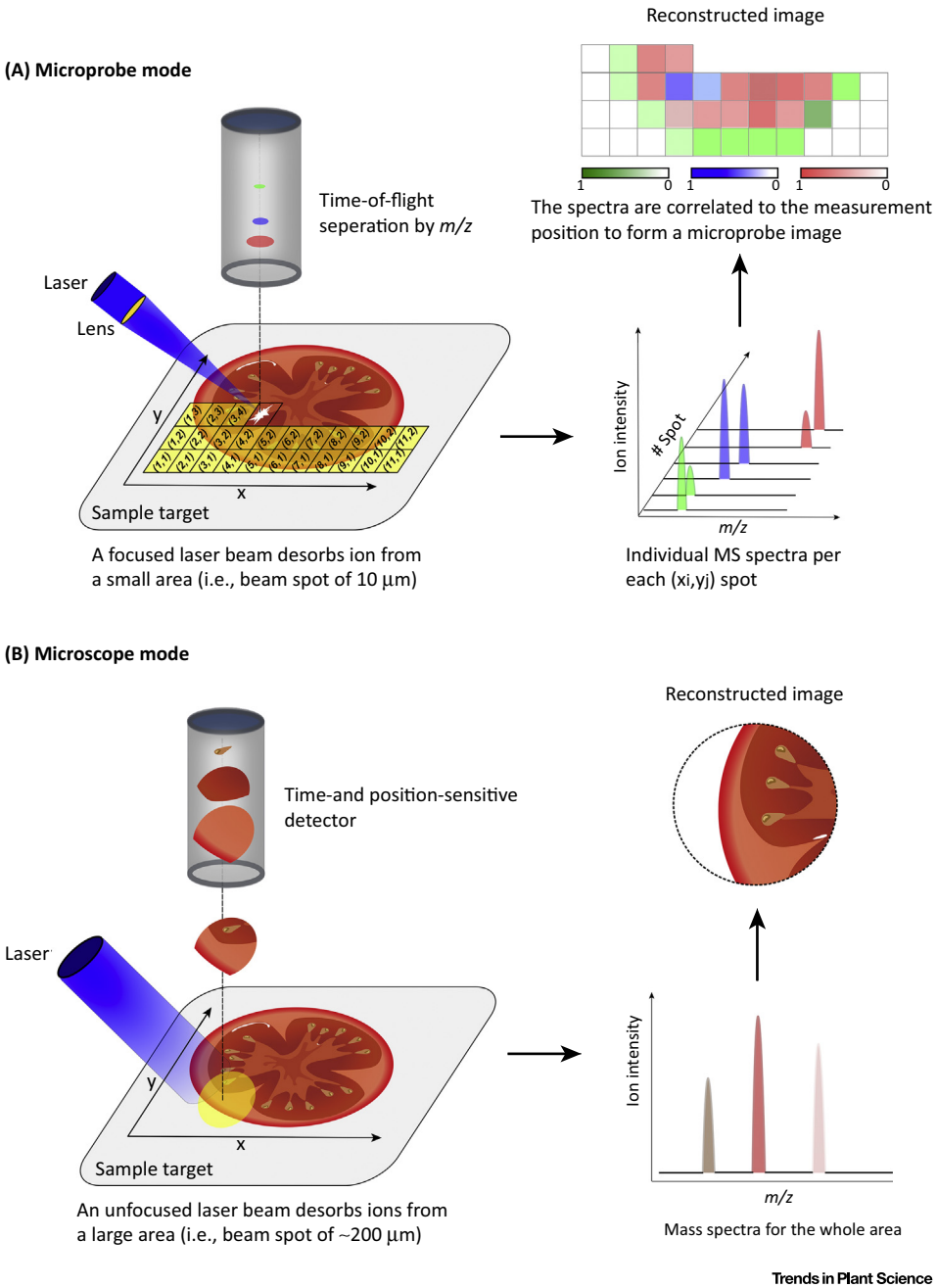

Figure 1. Schematic Overview of Microprobe and Microscope Mode Mass Spectrometry Imaging (MSI) Demonstrated on a Matrix-Assisted Laser Desorption Ionization (MALDI)-time-of-flight (TOF)/MS Platform. (A) In the microprobe mode MSI, a focused laser beam rasters the sample surface according to a predefined *x*, *y* grid. At each discrete spot, a mass spectrum is generated. An image with a pixel resolution equivalent to the beam size is reconstructed after the experiment. (B) In the microscope mode MSI, a broadly focused laser scans wide areas of the sample surface. Ion optics magnifies the molecular images and retains the spatial information. The molecular ion distributions are mapped on a position-sensitive detector.

Glossary

Depth resolution: a spatial resolution parameter used in 3D MSI. In the serial section-based 3D MSI, it refers to the tissue section thickness. In depth profiling-based 3D MSI, it refers to the profiling depth.

Fluorescence-activated cell sorting (FACS): a separation technique that enables a pool of different cells to be sorted one by one into one or more containers. The cells are typically sorted according to their specific light scattering and fluorescent characteristics.

Ion-mobility spectrometry (IMS): an analytical technique that separates gas-phase ions based on their size (*m/z*) and shape, analogous to electrophoresis in the condensed phase.

Laser-capture microdissection (LCM): an isolation technique that combines microscopy with laser beam technology, allowing for the isolation of target cells or tissue regions that need to be separated from others.

Mass:charge ratio (*m/z*): a value used in mass spectrometry obtained by dividing the mass of an ion by its charge number. For example, if an ion has a mass of 100 and a charge number of 1, its *m/z* is 100. An ion with a mass of 200 and a charge number of 2 also has an *m/z* of 100.

Mass spectrometer: an analytical instrument capable of ionizing analytes, separating them based on their *m/z*, and detecting them to produce a mass spectrum.

Reactive MSI: an MSI analysis approach where a compound that is capable of selectively reacting with analyte of interest is added into MSI (e.g., in the MALDI matrix or DESI spray solvent), allowing one to monitor the product of the reaction. This is usually carried out to increase the ionization yield of the target analyte or to isolate a specific isomer from a family of compounds.