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Review

Imaging lipids with secondary ion mass spectrometry ☆

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ABSTRACT

This review discusses the application of time-of-flight secondary ion mass spectrometry (TOF-SIMS) and magnetic sector SIMS with high lateral resolution performed on a Cameca NanoSIMS 50(L) to imaging lipids. The similarities between the two SIMS approaches and the differences that impart them with complementary strengths are described, and various strategies for sample preparation and to optimize the quality of the SIMS data are presented. Recent reports that demonstrate the new insight into lipid biochemistry that can be acquired with SIMS are also highlighted. This article is part of a Special Issue entitled Tools to study lipid functions.

1. Introduction

The abundances and distributions of various lipid species within tissues and cells are linked to both health and disease [1–8]. In mammalian cells, lipids are not only the building blocks of cellular membranes, but they also function as ligands that selectively bind to and regulate the activity of certain protein components in signaling pathways [9–18]. Insight into lipid metabolism, transport, and function has been acquired by studying their distributions within tissues and cells. The locations and abundances of various lipid species are often probed by using fluorescent lipid analogs, lipophilic dyes, or lipid-specific functionalized antibodies that can be detected with light, fluorescence, or electron microscopy [19–23]. The distributions of various lipid species in biological samples can also be mapped with high chemical specificity and without the use of complex labels, such as fluorophores or antibodies, with imaging mass spectrometry.

Matrix-assisted laser desorption ionization (MALDI) is perhaps the most popular imaging mass spectrometry technique that has been used to analyze biological samples. As the name implies, the sample is coated with a matrix that promotes biomolecule desorption and ionization, and a laser is used to ionize the molecules within its focal area. This ionization process minimizes molecular fragmentation, enabling the detection of molecular ions with high mass-to-charge ratios (m/z 500–20,000) [24,25]. MALDI has been used to image a wide range of biomolecules, including lipids and proteins, with a lateral resolution that is typically >10 μm and a sampling depth in the micron range [25–30]. This spatial resolution renders MALDI imaging appropriate for analyzing the lipid distributions within tissues. MALDI imaging of biomolecules, including lipids, in tissues has been the subject of recent reviews [24,26,31–33] and will not be discussed further herein.

This review will focus on a complementary imaging mass spectrometry technique, secondary ion mass spectrometry (SIMS). SIMS offers higher spatial resolution than MALDI, but typically at the expense of higher molecular fragmentation, which results in lower chemical specificity. SIMS can achieve the sub-micrometer lateral resolution that enables studying the lipid distribution within a single cell. Moreover, the analysis depth of SIMS is ultimately limited by the escape depth of the secondary ions, which is generally limited to the upper one to two monolayers of the sample [34]. Thus, when performed at the surface of the sample, SIMS has the shallow sampling depth (top ≤ 5 nm) that permits imaging the lipids in the plasma membrane with little interference from intracellular membranes.

2. SIMS techniques

SIMS imaging of lipids has been performed using two different types of instrumentation, time-of-flight SIMS (TOF-SIMS) and magnetic

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sector SIMS with high lateral resolution. The basic principles that are common to both approaches will be presented first. Then the differences between the two approaches that impart them with distinctive strengths and weaknesses will be discussed.

Both TOF-SIMS and high-resolution magnetic sector SIMS are performed under ultra-high vacuum (UHV), which is the techniques' greatest limitation. During analysis, a focused primary ion beam sputters neutral and ionized molecules and molecular fragments from the surface of the sample. This primary ion beam is scanned across the sample, and the secondary ions that are ejected at each beam position are collected. The intensities of the secondary ions that are characteristic of specific components are then used to create a map of that component's distribution on the surface of the sample. Additionally, because material is sputtered from the surface of the sample during SIMS analysis, maps of component distribution at progressively increasing depth in the sample can be generated by repeatedly acquiring SIMS images at the same sample location. When depth profiling is performed with TOF-SIMS, a sputtering scan that removes the damaged material is often inserted after each imaging scan in order to reduce the fragmentation of the species detected during image acquisition.

The intensities of the secondary ions detected at each pixel are affected by the concentration of the parent molecule that produced the secondary ions, as well as by concentration-independent factors [35–43]. The first concentration-independent factor, which is referred to as matrix effects, arises because the local chemical environment affects the probability that a species will become ionized [35–40]. The second concentration-independent factor is sample topography; differences in the incidence angle of the primary ion beam affect both the absolute and relative intensities of the ions [41,42]. These concentration-independent variations in ion intensity complicate quantitatively imaging lipid distribution with either TOF-SIMS or high-resolution magnetic sector SIMS. Normalization methods have been developed to minimize these concentration-independent variations and produce a SIMS signal whose intensity is proportional to the concentration of the analyte. The different approaches that have been used to normalize TOF-SIMS and NanoSIMS data are described in the following section.

2.1. Comparison of TOF-SIMS and high-resolution magnetic sector SIMS

TOF-SIMS and high-resolution magnetic sector SIMS instruments differ in the configurations of their ion optics, primary ion sources, and mass analyzers (Table 1). These differences impart each approach with complementary strengths and weaknesses. In the following sections, these differences are discussed in the context of lipid imaging capabilities.

2.1.1. TOF-SIMS

TOF-SIMS instruments typically employ a pulsed primary ion beam that is oriented with oblique incidence to the plane of the sample [31]. The secondary ions that are ejected from the sample are then collected by a TOF mass analyzer that generates a mass spectrum at each pixel. The spectra can have a mass range of 1 to 1500 *m/z*, but the ion counts generally decrease with increasing mass due to fragmentation. Variations in the secondary ion signal intensity that are caused by matrix effects and sample topography can be reduced, but not eliminated, by normalizing the counts of the signal of interest to that of an abundant ion or all of the ions detected at the same pixel [44,45]. The collection of a mass spectrum at every pixel is advantageous because it negates the need for labels, as any unlabeled molecule that produces secondary ions with distinctive *m/z* can be detected and imaged. Other advantages are that multiple components of interest can be imaged in parallel, and the spectra contain information about both known and unknown components that are present in the sample.

The lateral resolution of any SIMS technique is ultimately limited by the diameter of the primary ion beam, which can be 200 nm or less, depending on the instrument. However, for imaging lipids with TOF-SIMS, the working lateral resolution is often larger than the beam diameter because the numbers of lipid-specific secondary ions detected at each beam position are insufficient for resolving features in an image [46]. Therefore, TOF-SIMS analyses must be optimized to increase the ejection of secondary ions that are characteristic of each lipid species of interest. Intact molecular ion species are most useful for this purpose. To maximize the detection of intact molecular ions and reduce the ejection of fragment ions from the sample, the amount of chemical damage at the surface of the sample must be minimized. Note that the term chemical damage is used to refer to molecular fragmentation, and does not refer to structural reorganization at length scales that are accessible to detection with SIMS. A common approach for minimizing molecular fragmentation is to limit the primary ion dose so that each primary ion impacts a pristine region on the sample, and not a region where the molecules were already fragmented by collision with a primary ion [47,48]. This is referred to as static SIMS analysis, where the static limit is typically estimated to be below 10^{13} ions/cm^2 [48,49].

Even when TOF-SIMS was performed under static conditions, molecular fragmentation can limit the selectivity and working lateral resolution of lipid imaging. When this occurs, fragment ions that are common to multiple species from a common lipid class, such as the phosphocholine headgroup-containing ions at *m/z* 184 $[C_5H_{15}NPO_4]^+$ and 224 $[C_8H_{19}NPO_4]^+$, can be detected to achieve a working lateral resolution ≤1 μm [45,50,51]. However, lipids from the same class cannot be discriminated by these fragment ions. Several strategies have been developed to increase the detection of secondary ion signals that are useful for lipid identification. The most effective approach to date is to

Table 1
Comparison of SIMS approaches.

	TOF-SIMS	NanoSIMS
Lateral resolution	≥500 nm	≥100 nm
Information	Molecular	Elemental & isotopic
Typical strategy for lipid identification	*m/z* of ions is characteristic to the component of interest	Isotope labeling is used to encode the lipid species of interest with a distinctive isotope signature
Primary ion source	Cluster ion sources (C_{60}^+, Bi_3^+, Au_3^+, SF_5^+)	Cs or oxygen
Mass analyzer	TOF	Magnetic sector
Secondary ion size	Molecular & high mass fragments	Monotomic & diatomic
Number of collected ions	Entire spectra	5–7 different *m/z* ratios
Requires a priori selection of target component(s)?	No	Yes
Unique capabilities	Identification of unknown lipid structures at specific locations in tissues; imaging unlabeled lipids with moderate (≥1 μm) lateral resolution	Imaging known lipids of interest within model or cellular membranes with high (~100 nm) lateral resolution
Major challenge	Low yields of intact molecular ions, interpretation of the mass spectra	Selective incorporation of distinct isotopes or elements into specific lipid species