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REVIEW ARTICLE

Lipid analysis in biological structures – Is time-of-flight secondary ion mass spectrometry a valuable tool in nano-lipidomics?

Magdalena Elżbieta Skalska[1,2ABCDEFGHIJKLMN]

[1]Department of Medical Physics, M. Smoluchowski Institute of Physics, Faculty of Physics, Astronomy and Applied Computer Science, Jagiellonian University, Kraków, Poland
[2]Centre for Theranostics, Jagiellonian University, Kraków, Poland

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CORRESPONDING AUTHOR:
Magdalena Elżbieta Skalska; Department of Medical Physics, M. Smoluchowski Institute of Physics, Faculty of Physics, Astronomy and Applied Computer Science, Jagiellonian University; Lojasiewicza street 11, 30-348 Kraków, Poland; E-mail: magda.skalska@uj.edu.pl

ABSTRACT

Introduction: Lipids are crucial biomolecules that confer structural integrity to cell membranes, facilitate signalling and regulate energy dynamics. Dysregulation of lipids is associated with various diseases, including diabetes, chronic inflammation, and neurological and cardiovascular disorders.
Objective: This review seeks to critically evaluate recent advancements in lipidomics, particularly concerning membranous nanoparticles such as extracellular vesicles (EVs), and to investigate the analytical potential of time-of-flight secondary ion mass spectrometry (ToF-SIMS) for nanoscale lipid mapping.
Methods: A comprehensive literature review was conducted, focusing on mass spectrometry (MS)-based lipidomic methodologies. Particular emphasis was placed on studies utilising ToF-SIMS to image lipid distribution and composition in cells and membrane-bound nanoparticles. While traditional MS techniques are proficient in identifying and quantifying lipids, they lack spatial resolution. ToF-SIMS addresses this limitation by enabling in situ molecular imaging at micrometre scales, revealing lipid heterogeneity within biological structures and providing unique insights into membrane architecture and lipid sorting. A comparative evaluation highlights both the strengths (e.g., spatial accuracy) and limitations (e.g., challenges in quantification) of ToF-SIMS in relation to alternative methods.
Conclusions: ToF-SIMS introduces a critical spatial dimension to lipidomics, bridging conventional bulk analysis with nano-lipidomic imaging. Its integration with complementary techniques holds promise for novel insights into lipid biology, biomarker discovery, and translational applications in diagnostics and drug delivery.

KEYWORDS

lipidomics, mass spectrometry, nanoparticles, extracellular vesicles, time of flight – secondary ion mass spectrometry

BRIEF DESCRIPTION OF THE WORK

This text discusses the use of ToF-SIMS to obtain important lipidomic information from nanometric structures, including extracellular vesicles. This proposed approach has the potential to help understand diseases at the molecular level. This review summarises different methods for analysing lipids in nanometric biostructures and outlines the requirements for further development of this approach. It also explores the potential advances and perspectives of ToF-SIMS in life science research, anticipating a significant impact in this area.

LIST OF ABBREVIATIONS

AD – Alzheimer's disease
APCI-MS – Atmospheric Pressure Chemical Ionisation Mass Spectrometry
APPI-MS – Atmospheric Pressure Photoionisation Mass Spectrometry
CE – Cholesteryl Esters
COVID-19 – coronavirus disease 2019
DAGs – diglycerides
DESI – desorption ESI
DIA – Data-Independent Acquisition
ELISA – enzyme-linked immunosorbent assays
ESI – electrospray ionisation
EVs – Extracellular Vesicles
FTIR – Fourier Transform Infrared Spectroscopy
HPLC – High-Performance Liquid Chromatography
HPTLC – High-Performance Thin-Layer Chromatography
LNPs – lipid nanoparticles
MALDI – Matrix-Assisted Laser Desorption / Ionisation
MS – Mass Spectrometry
NLCs – Nanostructured Lipid Carriers
NMR – Nuclear Magnetic Resonance
PC – phosphatidylcholine
PD – Parkinson's disease
POPC – 1-oleoyl 2-palmitoyl-sn-glycerol-3-phosphocholine
SLNs – Solid Lipid Nanoparticles
TAGs – triglycerides
TIC – Total Ion Chromatogram
TLC – Thin-Layer Chromatography
ToF-SIMS – Time-of-Flight Secondary Ion Mass Spectrometry
XIC – Extracted Ion Chromatogram

INTRODUCTION

Lipids

The significance of lipids in biological systems is becoming increasingly recognised in scientific research, as they play crucial roles in metabolism, signalling, and the development of diseases. No single, universal, and precise definition encompasses all lipid compounds [1]. Generally, lipids are a diverse group of naturally occurring biomolecules that are insoluble in water but soluble in nonpolar organic solvents, such as chloroform, hydrocarbons, alcohols, esters, and ethers [2, 3].

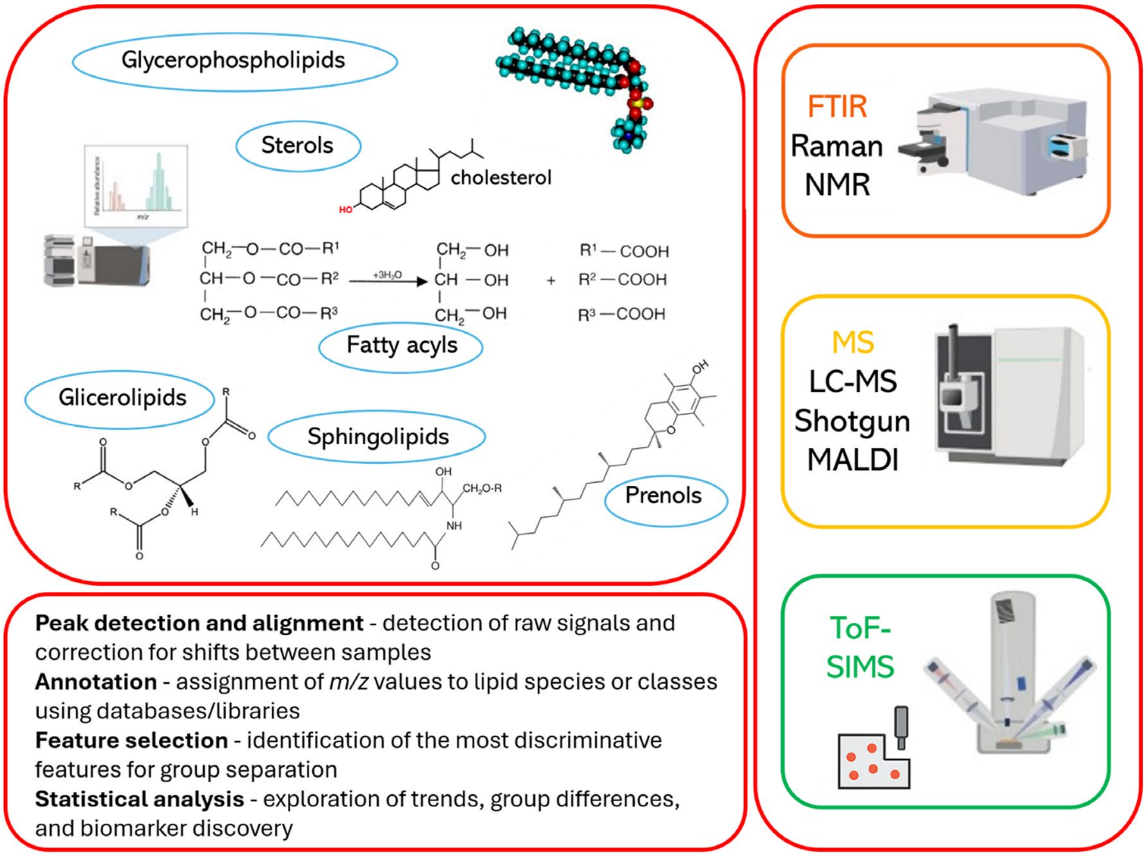
Eukaryotic cells contain thousands of distinct lipid species that fulfil multiple functions [4]. They serve as a condensed energy store in the form of triacylglycerols, act as precursors for signalling molecules, participate in intercellular communication, and function as both first and second messengers in intracellular signalling cascades [5, 6]. In plants and animals, lipids also play insulating and protective roles [7]. For instance, the lipid coat of mammalian skin contributes to homeostasis, including protection against inflammation, as well as participating in the immune response [8, 9].

One of the most fundamental roles of lipids is their contribution to the structure and function of biological membranes. Lipids determine the physical and chemical properties of membranes, and their precise composition is essential for physiological metabolism [10]. The lipid bilayer is the fundamental structure of cell and nanoparticle membranes, and its constituent lipids are amphipathic [4]. This structural characteristic classifies the lipids in the membrane into different categories, such as glycerolipids, glycerophospholipids, sphingolipids, and sterols (Fig. 1.). Variations in the number of carbon atoms and double bonds give rise to different species within each lipid class [11].

The two layers of the cellular membrane bilayer have an uneven lipid distribution [12]. For instance, in the plasma membrane most sphingolipids and phosphatidylcholine (PC) are found in the outer leaflet, whereas almost all other phospholipids are present in the cytosolic leaflet (phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol) [13]. There is a growing need to understand the intracellular distribution of various lipid classes, including the quantities of different species present in the various intracellular membranes. Gaining a deeper understanding of the intracellular distribution and species-level composition of lipids remains a significant challenge in cell biology.

Lipids in nano-sized biological structures

In recent years there has been growing interest in the lipid composition of nano-sized biological structures, such as lipid nanoparticles (LNPs) and extracellular vesicles (EVs). Lipid nanoparticles have become relevant in various fields, including drug delivery, medical imaging, cosmetics, nutrition, and agriculture [14, 15]. Their key advantages include biocompatibility, biodegradability, and the ability to encapsulate and transport molecules with a wide range of physicochemical properties, from hydrophobic lipids to hydrophilic proteins and nucleic acids [16, 17]. Advancements in formulation techniques have led to the development of solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs), which offer improved structural stability, controlled drug release, and enhanced encapsulation efficiency [18, 19]. Nevertheless, the clinical translation of synthetic lipid nanocarriers remains limited by challenges such



Peak detection and alignment - detection of raw signals and correction for shifts between samples
Annotation - assignment of *m/z* values to lipid species or classes using databases/libraries
Feature selection - identification of the most discriminative features for group separation
Statistical analysis - exploration of trends, group differences, and biomarker discovery

Fig. 1. Typical workflow for lipid biomarker identification.

as restricted bioavailability, potential toxicity, slow clearance, and immune activation [20, 21].

Simultaneously, research on naturally occurring nanoscale lipid assemblies, particularly EVs, has recently intensified. EVs are secreted by nearly all cell types under both physiological and pathological conditions. They exhibit high stability, low immunogenicity, biocompatibility, and efficient penetration of biological membranes, which makes them attractive candidates for diagnostics and therapeutics [22]. Based on size and biogenesis, EVs are classified into small EVs (exosomes, ~30–150 nm), large EVs (ectosomes, 100 nm–1 μm), and apoptotic bodies (50 nm–5 μm) [23]. Their bioactive cargo, including proteins, nucleic acids, metabolites, and lipids, reflects the state of the parent cell and can provide early molecular signatures of pathological processes, such as cancer, metabolic disorders, and neurodegenerative or cardiovascular diseases [24].

Comprehensive literature and database analyses indicate that EVs contain nearly 100,000 proteins, more than 1,000 lipid species, and numerous mRNAs and miRNAs [25, 26]. Their molecular composition varies with cell type and growth conditions, while pathological states modulate EV content, positioning EVs as informative reporters of nonstandard processes in biological systems [27]. Consequently, EVs have emerged as promising indicators of early pathological changes such as cancers, metabolic alterations and neurodegenerative or cardiovascular diseases [28–30], underscoring the need for noninvasive indicators to monitor diagnostic and therapeutic processes [31].

Importantly, EV lipidomes differ from those of their cells of origin. EVs are typically enriched in cholesterol, sphingomyelin, and saturated phospholipids, often exhibiting a higher lipid-to-protein ratio than parental membranes [24, 32]. These differences reflect selective lipid sorting during vesicle biogenesis and are crucial for vesicle stability, targeting, and uptake. However, comprehensive and sensitive quantification of lipids in EVs remains analytically challenging [33].

Emergence of lipidomics

The importance of lipids in health and disease has led to the emergence of a specialised field known as lipidomics [1, 34]. Lipidomics encompasses both the qualitative and quantitative analysis of lipids in biological systems, as well as the elucidation of their biological functions and interactions with genes and proteins involved in lipid metabolism [35, 36].

Today, it is possible to quantify approximately 1,000 of these in a sample using various analytical methods, such as mass spectrometry (MS) [37]. This relatively new area of research is being enriched with information obtained from modern research techniques derived from experimental physics, such as nuclear magnetic resonance (NMR) or fluorescence spectroscopy [38–41]. Increasing attention is being paid to the issue of the influence of the level of individual lipids as a factor implicated in and regulating the development of many diseases, such as obesity, circulatory system diseases, and some forms of cancer [42, 43].

Despite impressive progress, current lipidomic approaches often lack the spatial resolution necessary to characterise lipids in nanosized biological structures at the single-particle level. This limitation is now driving the emergence of nano-lipidomics, a subfield focused on the characterisation of lipid species in nanoscale systems, such as EVs, viral particles, and synthetic lipid nanoparticles. Here, advanced imaging mass spectrometry methods, including Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS), offer unique opportunities to overcome these challenges.

Analytical techniques in EV lipidomics

EVs circulating in the body are increasingly recognised as a potential source of biomarkers for diagnosing and tracking diseases. To understand their role as biomarkers, it is essential to understand their composition and monitor changes in this composition, along with the information they convey. While protein changes have been extensively studied, variations and modifications in lipid profiles can also offer important insights into the pathologies of biological systems.

The total lipid content of a cell or EVs is called lipidome, and the study of lipidomics, which involves understanding lipid metabolism pathways and related processes, has been a rapidly evolving field of research since 2003 [35, 44]. This area of science enables the identification of biochemical mechanisms associated with lipids, their functions in information transport, the indication of new metabolic pathways, and the detection of new indicators [45]. Additionally, it also helps in evaluating the effectiveness of pharmacological interventions [46].

Several studies highlight the clinical importance of EV lipidomics. For example, EVs derived from the frontal cortex of Alzheimer's disease (AD) patients are enriched in glycerol-phospho-ethanolamine and polyunsaturated fatty acyl-containing lipids. Meanwhile, astrocyte-derived EVs in amyloid pathology contain ceramide species that may contribute to disease progression [47]. Some other examples include cardiovascular diseases [48], Parkinson's disease (PD) [49], various cancers [50], and kidney disease [51]. Currently, there is limited knowledge regarding the lipid composition of circulating EVs. As many of these lipid components exhibit biological activity, gaining a better understanding of the lipid components of EVs isolated from serum samples will be beneficial for discovering new biomarkers.

Sample handling and extraction

The accuracy and quality of lipid determination depend on several factors that must be considered before analysis. Both the type and quantity of samples influence these factors. The main parameters affecting lipid determination include the method of lipid extraction from the sample, the selection of appropriate internal standards, and the conditions and duration of sample storage (Fig. 2.) [52, 53]. Lipids are susceptible to oxidation of unsaturated fatty acyl chains. Therefore, adding antioxidants and storing extracts at −80°C under inert gas, such as argon, significantly improves their stability [54].

When determining the composition and lipid content of isolated EV samples, it's important to be aware of potential contamination from lipid droplets and lipoproteins. These contaminants are rich in triacylglycerols (TAG) and cholesteryl esters (CE) and are similar in size to EVs. This can be assessed by incorporating

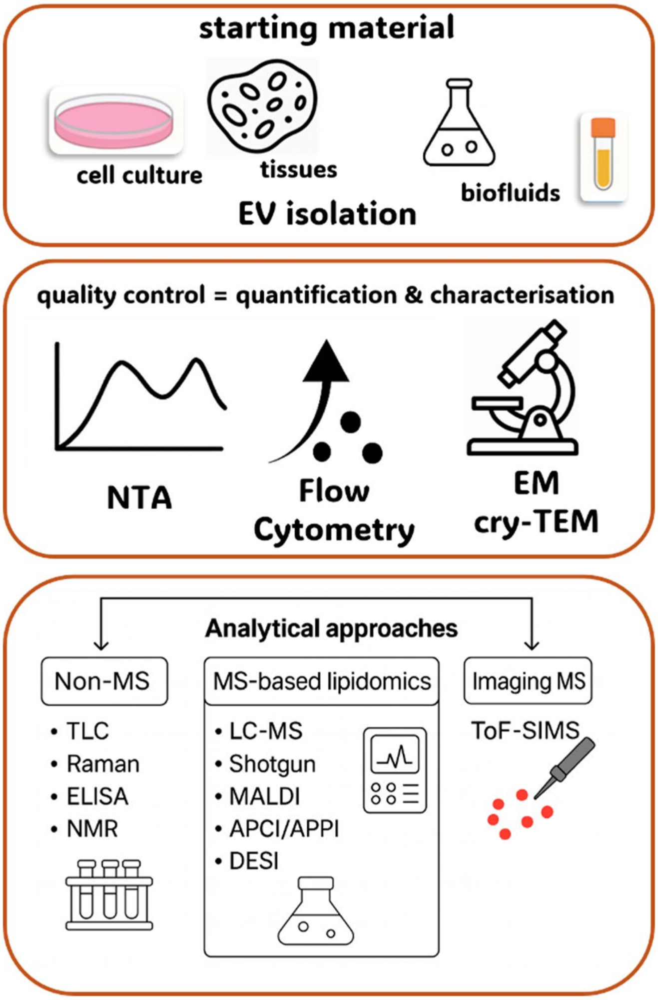


Fig. 2. Analytical pipeline for EV lipidomics.

substances that stain lipid droplets into the preparations [55]. Therefore, omitting fetal calf serum added to the cell culture early enough before EV isolation should also be considered, as it contains lipid molecules of similar size to EVs, and the lipid composition of the growth medium and lipids added to the medium influence the lipid content of the cells and the EVs they release [56].

The results of lipidomic analyses can be expressed as either absolute quantitative values (in the form of pmol/μg protein) or relative values for different classes or species. These values demonstrate changes under various culture conditions or cell treatments. Importantly, having quantitative data available enables other researchers to evaluate the purity of samples and the quality of the data obtained [57].

Conventional methods

From the early stages of studying EVs' lipid composition, MS has played a crucial role in the qualitative and quantitative characterisation of lipids, establishing itself as the most effective approach in this field. Recent advancements in lipid extraction and analysis, combined with bioinformatics, have significantly propelled lipidomics research forward [58].

Although mass spectrometry has become the central platform for lipidomic studies, a variety of conventional and complementary techniques continue to contribute valuable information to EV lipid analysis (Tab. I.). One of the earliest approaches was thin-layer chromatography (TLC), which allowed researchers to demonstrate differences in the lipid composition between exosomes and their parental cells [59]. Refinements in this methodology led to high-performance thin-layer chromatography (HPTLC), which provided more reproducible and faster analysis of phospholipids in EVs derived from fibroblasts and melanoma cells [60]. Despite limited resolution compared to modern mass spectrometry, TLC based methods remain useful for preliminary profiling or as confirmatory techniques.

Raman spectroscopy has also been increasingly applied in EV research. When combined with comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC × GC–TOFMS), Raman spectroscopy has been used to distinguish fatty acid compositions between EV subpopulations isolated from human plasma, differentiating between esterified membrane lipids and non-esterified fatty acids [61]. Furthermore, Raman-based analyses have revealed the presence of amyloid-β peptide signatures in EVs derived from midbrain organoids, underscoring the potential of vibrational spectroscopy for identifying disease-associated molecular fingerprints [62]. Other biochemical assays, including enzyme-linked immunosorbent assays (ELISA), colourimetric reactions such as sulfo-vanillin, and lipid-specific fluorescent dyes, have been employed for the quantification of total lipid content and protein-to-lipid ratios [59, 63]. While these approaches offer relatively simple workflows, their applicability is limited by low specificity and poor molecular resolution. Fourier transform infrared spectroscopy (FTIR) has been proposed as another bulk lipid quantification method, although it similarly lacks the capacity to resolve individual lipid classes.

NMR spectroscopy represents a more sophisticated non-destructive alternative. Techniques such as ^{1}H DOSY and ^{129}Xe Hyper-CEST NMR have been employed to investigate EV dynamics, size distribution, and heterogeneity among subpopulations [64]. Importantly, integration of NMR with complementary methods has enabled deeper insights into EV lipidomes. For example, a combined LC-MS/MS and ^{31}P NMR study of plasma-derived exosome-like vesicles showed enrichment in lysophospholipids and absence of phosphatidylserine, in stark contrast to cell culture-derived EVs [65]. Collectively, these non-MS methods provide valuable contextual information and can serve as confirmatory or complementary tools, but they lack the sensitivity, specificity, and throughput required for comprehensive lipidomic profiling.

Mass spectrometry

MS is widely recognised as the leading technique for lipid analysis, offering well-developed methods for sample and data processing, as well as comprehensive insights into lipid components [66]. The success of MS-based lipidomics depends not only on sensitive instrumentation but also on robust extraction and pre-analytical workflows. Following isolation, lipids are typically fractionated by physical approaches, such as liquid–liquid partitioning or solid-phase extraction to separate polar from nonpolar fractions [53]. Alternatively, chemical methods such as base hydrolysis can be employed to enrich low-content sphingolipids from complex lipid extracts that contain high concentrations of phospholipids and glycerolipids [57]. Another approach involves

Tab. I. Non-MS techniques for EV lipidomics.

METHOD	APPLICATION / INSIGHT	EXAMPLE SYSTEM (REF.)
TLC / HPTLC	phospholipid composition, enrichment vs. parental cells	fibroblast-derived EVs, melanoma-derived EVs [68, 69]
Raman spectroscopy	fatty acid composition and amyloid-β fingerprint	plasma-derived EVs [66]; midbrain organoids [67]
ELISA / dyes / FTIR	total lipid content; protein-to-lipid ratios	various EV samples [68, 71, 72]
NMR	lipid dynamics, lysophospholipid enrichment	plasma-derived EVs [73, 74]

derivatising extracts by chemically labelling specific functional groups of lipids, which is beneficial for analysing lipids that lack inherently charged fragments or do not have characteristic fragmentation patterns during tandem mass spectrometry (MS/MS) analysis [67].

Two main MS strategies dominate the field of lipidomics. Chromatography-based lipidomics, particularly liquid chromatography coupled to mass spectrometry (LC–MS), enables both targeted and untargeted analyses. The incorporation of data-independent acquisition (DIA) workflows has further strengthened LC–MS, allowing simultaneous MS/MS fragmentation of all precursor ions and thus broadening the scope of untargeted lipidomics [67]. Conversely, shotgun lipidomics eliminates the need for chromatographic separation, utilising direct infusion techniques such as flow injection. This approach offers high-throughput profiling of hundreds of lipid species but may be affected by ion suppression and limited resolution of isobaric species.

In MS, the first step is ionisation, which is crucial for the interpretation of mass spectra. Several ionisation techniques are commonly used in modern mass spectrometry for lipidomics, with the choice of technique depending on experimental goals and sample characteristics. One of the most popular methods is electrospray ionisation (ESI). ESI is a type of soft ionisation that involves applying a strong electric field to a liquid flowing through a capillary tube, resulting in the generation of an electrospray. Ions are produced from fine aerosols through desolvation. Coupling ESI with tandem mass spectrometry (ESI-MS/MS) helps overcome the limitations of obtaining very little structural information from the simple ESI mass spectrum. ESI can also be coupled with high-performance liquid chromatography (HPLC) for the analysis of both small and large molecules [68]. For example, it has been used to quantitatively profile the lipidome of exosome-enriched EVs during COVID-19, with a focus on various oxidised derivatives of cholesterols and phytosterols that are known to elicit immuno-modulatory and antiviral effects [69].

Matrix-assisted laser desorption / ionisation (MALDI) is also a soft ionisation technique used to analyse large and/or unstable molecules, such as lipids. This technique involves depositing analytes in a matrix that absorbs energy at the laser's wavelength. A pulsed laser illuminates the analytes, causing the ablation and desorption of both the analytes and the matrix material. This process facilitates the ionisation of analyte molecules in a hot plume of ablated gases. MALDI-MS is a powerful tool for the analysis and characterisation of EVs' lipids, and their cargo [70]. This technique offers advantages such as a high throughput, minimal sample consumption, rapid and cost-effective analysis and user-friendly operation [71]. This technique was used to prove EV lipid changes in pathological states such as melanoma and LIM1215 colorectal cancer cells [72, 73].

Atmospheric pressure chemical ionisation mass spectrometry (APCI-MS) is a soft ionisation method that uses gas-phase ion-molecule reactions at atmospheric pressure. It can ionise various lipids, including protonated molecular ions of phospholipids, free fatty acids and sterols. Ionisation occurs along the corona discharge electrode, where the relative proton affinity of the reactant gas ions and the gaseous analyte molecules allows for proton transfer, abstraction or adduct formation to produce molecular ions [74].

Atmospheric pressure photoionisation mass spectrometry (APPI-MS) can be utilised to analyse neutral lipids and phospholipids with higher sensitivity and lower detection limits compared to APCI-MS [75]. This technique involves the use of a vacuum-ultraviolet lamp specifically designed for photoionisation detection in gas chromatography. The sample-solvent mixture evaporated and was then induced into the photoionisation region, where the dopant photoions react completely with the solvent and analyte molecules [37].

In desorption ESI (DESI), ionisation occurs by directing a charged electrospray mist to the sample surface. Subsequently, splashed droplets carry desorbed and ionised analytes that travel into the mass spectrometer's atmospheric pressure interface. This technique has been effectively used to characterise phospholipids systematically [76]. Before conducting an MS analysis, the ion mobility technique (IM-MS) can be applied immediately after ionisation to manipulate and separate ions further [77]. Following the MS analysis, the data are presented as MS spectra, MS/MS spectra, and an ion chromatogram, which includes a total ion chromatogram (TIC) or extracted ion chromatogram (XIC).

Over time, advancements in ionisation techniques have led to the adoption of new methods for directly characterising lipids in membranous structures. One such direct technique is time-of-flight secondary ion mass spectrometry (TOF-SIMS). Initial approaches to analysis used silver or gold ions as primary ions. However, the technique later evolved to use heavier ion clusters, which improved the emission efficiency of larger parent ions.

ToF-SIMS compared with other techniques

While conventional lipidomic methods and mass spectrometry workflows have provided valuable insights into the lipid composition of EVs and other nanoscale structures, a major limitation remains – the loss of spatial information due to lipid extraction. In most analytical strategies lipids are removed from their native environment, which eliminates crucial data regarding their localisation within membranes or vesicular substructures. However, knowledge of lipid distribution is essential for understanding functional membrane organisation and the molecular mechanisms underlying disease pathology [78].

ToF-SIMS addresses this limitation by enabling direct in situ analysis of intact biological surfaces. In ToF-SIMS, a focused beam of primary ions bombards the sample, producing secondary ions that are analysed according to their mass-to-charge ratio. This technique enables the simultaneous acquisition of mass spectra, two-dimensional chemical maps, and depth profiles, which cover the outermost atomic layers of the sample [79].

ToF-SIMS achieves lateral resolutions of up to 50–60 nm, allowing for detailed visualisation of lipid domains in subcellular and nanoscale structures [80]. Due to its versatile capabilities, ToF-SIMS has been widely used in various fields such as materials science, surface chemistry, biotechnology, and nanotechnology [81]. In recent years it has become a valuable tool for studying biological systems, including imaging biological cells and tissues, as well as analysing proteins and lipids. Advancements in ion sources, sample preparation techniques, and data analysis programs have further broadened the applications of ToF-SIMS [82].

The initial biological applications of ToF-SIMS focused on lipids with high secondary ion yields, such as cholesterol, vitamin E, and fatty acids. Researchers concentrated on these compounds due to their high ion emission efficiency [83]. In contrast, phospholipids and sphingolipids require accumulation in specific areas to be detectable in mass spectra because they exhibit lower signal intensity. Since the inception of ToF-SIMS, a comprehensive library of spectra and an extensive list of characteristic peaks in lipid mass spectra have been developed [84].

One significant study was conducted by McMahon in 1995, which analysed phosphatidylcholine and sphingomyelin in pig brain tissues and adrenal gland samples from dogs [85]. Another notable example is the identification of cholesterol in the blood of individuals with Smith-Lemli-Opitz syndrome, making the beginning of the use of this method in biomedicine [86]. In this field, high-resolution 2D maps of cholesterol redistribution in the human intestine after prior administration of cholera toxin have been obtained. These studies demonstrated an increased accumulation of cholesterol in the enterocytes of intestinal villi in the tissues treated with the toxin [87]. Subsequent research has shown specific accumulation of two types of glycosphingolipids in skin and kidney samples from patients diagnosed with Fabry disease. These lipids were found to accumulate in the dermis and subcutaneous tissue, while healthy volunteers showed weak signals from lipids in the epidermis. Additionally, in kidney biopsy materials a correlation was observed between the intensity level of peaks corresponding to glycosphingolipids and the stage of disease progression [88].

The use of ionic conglomerates, such as Au_n+, Bi_n+, and C_{60}+, resulted in increased emission of secondary ions with higher masses, while simultaneously increasing the emission of parent molecular ions [89]. This advancement facilitated the analysis and three-dimensional visualisation of complex biological molecules. Ion beam conglomerates enabled the imaging of cholesterol in cells by Nygren et al., and helped in the identification of cholesterol, sulfatides, phosphatidylinositol, and phosphatidylcholine, as well as determining the localisation of cholesterol and phosphocholine in the rat cerebellar cortex [90]. By using a gold cluster (Au_3+) emitting gun, three different regions were distinguished in mouse leg samples based on the specific distribution of different lipid classes (fatty acids, triglycerides, phospholipids, tocopherol, coenzyme Q9 and cholesterol). These studies showed the possibility of direct localisation of a specific amount of lipids in the tissue, the content of which may reflect the metabolic state of the structure [91].

Attempts were also made to conduct quantitative studies. Prinz et al., by analysing different thicknesses of model phospholipid layers, showed that peaks assigned to ionised molecules, such as 1-oleoyl 2-palmitoyl-sn-glycerol-3-phosphocholine (POPC) dimer, have different intensities depending on the thickness of the layers of lipids studied [92]. They suggested that the occurrence of peaks indicating the emission of molecules can be used as a sensitive indicator of changes in membrane structures and provide information about the existence of bilayer membrane structures in cells and tissues. In the work of Ostowski et al., single cells were distinguished based on lipid composition, specifically using the example of cholesterol in the outer layers of the plasma membrane of macrophages [93].

Touboul and Kollmer conducted studies on the antioxidant properties of vitamin E and performed a detailed analysis of tocopherol localisation in the mouse brain [94]. Monroe also studied the same lipid and presented the localisation of tocopherol on a subcellular scale in the membranes of single, isolated neurons from the model *Aplysia californica* [95]. Additionally, the imaging of fatty acids in the rat retina, phosphatidic acids in the atherosclerotic plaque, and di- and triglycerides (DAGs, TAGs) in human adipose tissue was also performed [96]. In a landmark study, Song et al. combined ToF-SIMS imaging with immunofluorescence to distinguish different muscle fibre types in situ by mapping their unique metabolite signatures – specifically showing elevated levels of unsaturated diacylglycerols, oleic acid, and linoleic acid in Type I and IIA fibres compared to Type IIB [97]. In a complementary investigation, Marzec et al. utilised ToF-SIMS to spatially resolve fatty acid distributions in broiler breast muscle, revealing that dietary supplementation with various vegetable oils significantly alters intramuscular lipid profiles, including the ω6 / ω3 ratio, in both muscle fibres and intramuscular fat [98]. Together, these studies underscore ToF-SIMS's dual utility – as both an exploratory discovery platform and a targeted method for hypothesis-driven inquiries within metabolomics and nano-lipidomics.

Compared with other lipidomic methods, ToF-SIMS offers a unique balance of molecular specificity and nanoscale spatial resolution. While chromatographic MS and shotgun approaches remain superior for comprehensive quantification, ToF-SIMS excels at correlating lipid identity with precise localisation, which is critical for studying membrane organisation, vesicle heterogeneity, and nanoscale pathology. Current challenges include the complexity of sample preparation, the need for standardised spectral libraries,

Tab. II. ToF-SIMS vs. other techniques.

FEATURE	CONVENTIONAL MS (LC–MS, SHOTGUN)	IMAGING MS (MALDI, DESI)	TOF-SIMS
Sample preparation	Requires extraction; loss of spatial information	Requires matrix / coating, mild preparation	Minimal preparation, direct analysis
Spatial resolution	None (bulk quantification)	10–50 μm (tissue imaging)	50–60 nm (subcellular domains)
Information gained	Quantitative lipidome profiles	Tissue-scale maps; quantification	Molecular recognition with nanoscale localisation
Ionisation method	ESI / APCI / APPI, soft ionisation	Laser ablation or spray ionisation	Primary ion bombardment
Strengths	Broad coverage, robust quantification	High-throughput, tissue mapping	Nano-resolution maps; label-free analysis
Limitations	Loss of localisation; ion suppression	Limited to μm-scale; matrix effects	Semi-quantitative; complex data analysis

and the difficulty of achieving absolute quantification due to variable ion yields (Tab. II.). Nevertheless, advances in cluster ion technology, cryogenic preparation methods, and machine-learning–based data interpretation are rapidly expanding the analytical power of ToF-SIMS, positioning it as a uniquely valuable tool for nano-lipidomics [99].

ToF-SIMS in bio-nanoparticle research

Due to its high spatial resolution and chemical specificity, ToF-SIMS has emerged as a valuable technique in nanotechnology, particularly for biological and medical applications. It is especially effective in analysing the surface composition of nanoparticles and biomolecules, making it a powerful tool for advancing our understanding of nano-biotechnology and nanomedicine [100, 101].

One significant advantage of ToF-SIMS is its proficiency in single-cell imaging, enabling researchers to visualise the subcellular distribution of both endogenous and exogenous substances, including metallodrugs. This capability is critical for drug evaluation and understanding drug metabolism at the cellular level [102]. Numerous studies have demonstrated that this technique can visualize amino acids, lipids, sugars, and nucleotides directly within their native microenvironments [103].

A particularly compelling avenue lies in the lipidomic profiling of EVs. Compared to donor cells, EVs often exhibit elevated levels of cholesterol, sphingomyelin, and gangliosides, accompanied by a relative decrease in phosphatidylcholine and diacylglycerols [104]. These compositional distinctions reflect selective lipid sorting during EV biogenesis, imbuing vesicles with unique structural and functional membrane properties [105].

ToF-SIMS also offers powerful capabilities for interrogating nanoparticle–bio-interactions. In physiological fluids, nanoparticles rapidly acquire a "protein corona" – a layer of adsorbed proteins and lipids that modifies their biological identity. ToF-SIMS enables detailed molecular characterisation of this corona, offering insights into how it shapes nanoparticle uptake, biodistribution, and immunogenicity [24]. At the cellular level, ToF-SIMS has been used to visualise lipid and protein adsorption onto nanoparticle surfaces and to map their intracellular trafficking into organelles, such as endosomes or nuclei, thereby elucidating nanocarrier bio-compatibility and dynamics [106]. Moreover, ToF-SIMS enables the direct tracking of nanoparticles within tissue contexts and the monitoring of carrier degradation and active agent release. For example, Belu et al. [107] demonstrated that ToF-SIMS imaging enabled the in situ identification of drugs, coating materials, and excipients across multiple layers of controlled-release beads, offering insights into both the structural integrity and time-release mechanisms of these delivery vehicles.

These applications collectively underscore the substantial benefits of ToF-SIMS in nanobiological research, including its unparalleled surface sensitivity, nanoscale spatial resolution for the localisation of lipids and metabolites, and its depth profiling capability for reconstructing three-dimensional chemical maps. Despite these advantages, several challenges persist, including stringent sample preparation requirements to prevent contamination, the complexity of interpreting overlapping biological spectra, and the difficulty in achieving absolute quantification due to variable ion yields.

Ongoing innovations, such as cluster ion sources, cryogenic sample handling, and advanced computational analyses, including machine learning for spectral deconvolution, are anticipated to enhance the sensitivity, resolution, and interpretability of ToF-SIMS.

The integration of ToF-SIMS with complementary imaging modalities, such as fluorescence and electron microscopy, promises to establish a robust, multimodal framework for investigating the behaviour of bio-nanoparticles. These advancements position ToF-SIMS as an indispensable analytical technique in nano-lipidomics, capable of delivering comprehensive molecular characterisation of EVs, liposomes, and other bio-nanoparticles across both physiological and pathological contexts.

CONCLUSIONS

Over the past two decades, lipidomics and metabolomics have matured into powerful disciplines for dissecting the complexity of cellular regulation and disease. The next frontier lies in integrating these approaches with other omics layers – genomics, proteomics, and phosphor-proteomics – underpinned by expanding databases and standardised workflows that ensure reproducibility and comparability. Within this rapidly evolving field, ToF-SIMS is emerging as a uniquely powerful tool. Unlike conventional lipidomics, it preserves spatial information and enables direct in situ molecular mapping at the nanoscale. Recent studies demonstrate its ability not only to identify lipid species in cells, tissues, and extracellular vesicles but also to visualise their organisation, heterogeneity, and pathological redistribution.

Looking forward, three key developments are expected to shape the future of ToF-SIMS in nano-lipidomics. First, its integration with single-cell omics platforms will connect nanoscale lipid maps with transcriptomic, proteomic, and epigenomic profiles, offering a multimodal view of cellular heterogeneity. Second, the application of artificial intelligence and machine learning will accelerate spectral deconvolution and image interpretation, advancing the discovery of subtle lipid signatures and biomarkers. Third, cryogenic ToF-SIMS workflows will preserve native biological structures and enable high-fidelity analysis of hydrated and delicate samples, such as extracellular vesicles and organelles.

Collectively, these advances position ToF-SIMS as a cornerstone of nano-omics and translational biomedicine. Its integration with complementary imaging modalities promises to provide unprecedented insights into biomembrane organisation, vesicle biology, and nanoparticle behaviour, establishing ToF-SIMS as an indispensable platform for both fundamental research and clinical applications in diagnostics, drug delivery, and nanomedicine.

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REFERENCES

1. Navas-Iglesias N, Carrasco-Pancorbo A, Cuadros-Rodríguez L. From lipids analysis towards lipidomics: a new challenge for the analytical chemistry of the 21st century. Part II: Analytical lipidomics. Trends Anal Chem. 2009 Apr;28(4):393–403. doi: https://doi.org/10.1016/j.trac.2008.12.004.
2. Christie WW. Lipid analysis: isolation, separation, identification, and structural analysis of lipids. 2nd ed. Oxford: Pergamon Press; 1982.
3. Kates M. Techniques of lipidology: isolation, analysis and identification of lipids. 2nd rev ed. Amsterdam: Elsevier; 1986.
4. Cooper GM. The Cell: A Molecular Approach. 2nd ed. Sunderland (MA): Sinauer Associates; 2000.
5. Johansson B. ToF-SIMS imaging of lipids in cell membranes. Surf Interface Anal. 2006 Nov;38(11):1401–12. doi: https://doi.org/10.1002/sia.2403.
6. Fernandis AZ, Wenk MR. Membrane lipids as signaling molecules. Curr Opin Lipidol. 2007 Apr;18(2):121–8. doi: https://doi.org/10.1097/MOL.0b013e328082e4d5.
7. Stryer L. Biochemia. Warszawa: PWN; 2003.
8. McCusker MM, Grant-Kels JM. Healing fats of the skin: the structural and immunologic roles of the omega-6 and omega-3 fatty acids. Clin Dermatol. 2010 Jul–Aug;28(4):440–51. doi: https://doi.org/10.1016/j.clindermatol.2010.03.020.
9. Nicolaou A. Eicosanoids in skin inflammation. Prostaglandins Leukot Essent Fatty Acids. 2013 Jan;88(1):131–8. doi: https://doi.org/10.1016/j.plefa.2012.04.005.
10. van Meer G, Voelker DR, Feigenson GW. Membrane lipids: where they are and how they behave. Nat Rev Mol Cell Biol. 2008 Feb;9(2):112–24. doi: https://doi.org/10.1038/nrm2330.
11. Holthuis JCM, Levine TP. Lipid traffic: floppy drives and a superhighway. Nat Rev Mol Cell Biol. 2005 Mar;6(3):209–20. doi: https://doi.org/10.1038/nrm1591.
12. Fadeel B, Xue D. The ins and outs of phospholipid asymmetry in the plasma membrane: roles in health and disease. Crit Rev Biochem Mol Biol. 2009;44(5):264–77. doi: https://doi.org/10.1080/10409230903193307.
13. Skotland T, Hessvik NP, Sandvig K, Llorente A. Exosomal lipid composition and the role of ether lipids and phosphoinositides in exosome biology. J Lipid Res. 2019 Jan;60(1):9–18. doi: https://doi.org/10.1194/jlr.R084343.
14. Viegas C, Seck F, Fonte P. An insight on lipid nanoparticles for therapeutic proteins delivery. J Drug Deliv Sci Technol. 2022;77:103839. doi: https://doi.org/10.1016/j.jddst.2022.103839.
15. Mehta M, Bui TA, Yang X, Aksoy Y, Goldys EM, Deng W. Lipid-based nanoparticles for drug/gene delivery: an overview of the production techniques and difficulties encountered in their industrial development. ACS Mater Au. 2023 Nov;3(6):600–19. doi: https://doi.org/10.1021/acsmaterialsau.3c00029.
16. Wang EC, Wang AZ. Nanoparticles and their applications in cell and molecular biology. Integr Biol (Camb). 2014 Jan;6(1):9–26. doi: https://doi.org/10.1039/c3ib40165k.
17. Ghasemiyeh P, Mohammadi-Samani S. Solid lipid nanoparticles and nanostructured lipid carriers as novel drug delivery systems: applications, advantages and disadvantages. Res Pharm Sci. 2018 Aug;13(4):288–303. doi: https://doi.org/10.4103/1735-5362.236858.
18. López KL, Ravasio A, González-Aramundiz JV, Zacconi FC. Solid lipid nanoparticles and nanostructured lipid carriers prepared by microwave and ultrasound-assisted synthesis: promising green strategies for the nanoworld. Pharmaceutics. 2023 May;15(5):1343. doi: https://doi.org/10.3390/pharmaceutics15051343.
19. Khosa A, Reddi S, Saha RN. Nanostructured lipid carriers for site-specific drug delivery. Biomed Pharmacother. 2018 May;103:598–613. doi: https://doi.org/10.1016/j.biopha.2018.04.055.
20. Dhiman N, Awasthi R, Sharma B, Kharkwal H, Kulkarni GT. Lipid nanoparticles as carriers for bioactive delivery. Front Chem. 2021;9:691136. doi: https://doi.org/10.3389/fchem.2021.691136.
21. Mirahadi M, Ghanbarzadeh S, Ghorbani M, Gholizadeh A, Hamishehkar H. A review on the role of lipid-based nanoparticles in medical diagnosis and imaging. Ther Deliv. 2018 Aug;9(8):557–69. doi: https://doi.org/10.4155/tde-2018-0011.

22. Ghadami S, Dellinger K. The lipid composition of extracellular vesicles: applications in diagnostics and therapeutic delivery. Front Mol Biosci. 2023 Jul;10:1208161. doi: https://doi.org/10.3389/fmolb.2023.1208161.
23. Doyle L, Wang M. Overview of extracellular vesicles, their origin, composition, purpose, and methods for exosome isolation and analysis. Cells. 2019 Jul;8(7):727. doi: https://doi.org/10.3390/cells8070727.
24. Skalska ME, Durak-Kozica M, Stępień EŁ. ToF-SIMS revealing sphingolipids composition in extracellular vesicles and paternal β-cells after persistent hyperglycemia. Talanta. 2026 Jan;297:128582. doi: https://doi.org/10.1016/j.talanta.2025.128582.
25. Yáñez-Mó M, Siljander PRM, Andreu Z, Zavec AB, Borràs FE, Buzas EI, et al. Biological properties of extracellular vesicles and their physiological functions. J Extracell Vesicles. 2015 May;4:27066. doi: https://doi.org/10.3402/jev.v4.27066.
26. Kim DK, Lee J, Kim SR, Choi DS, Yoon YJ, Kim JH, et al. EVpedia: a community web portal for extracellular vesicles research. Bioinformatics. 2015 Mar 15;31(6):933–9. doi: https://doi.org/10.1093/bioinformatics/btu741.
27. Kumar MA, Baba SK, Sadida HQ, Al Marzooqi S, Jerobin J, Altemani FH, et al. Extracellular vesicles as tools and targets in therapy for diseases. Signal Transduct Target Ther. 2024;9(1):27. doi: https://doi.org/10.1038/s41392-023-01726-1.
28. Raghav A, Singh M, Jeong GB, Giri R, Agarwal S, Kala S, et al. Extracellular vesicles in neurodegenerative diseases: a systematic review. Front Mol Neurosci. 2022;15:1061076. doi: https://doi.org/10.3389/fnmol.2022.1061076.
29. Zhang Y, Liang F, Zhang D, Qi S, Liu Y. Metabolites as extracellular vesicle cargo in health, cancer, pleural effusion, and cardiovascular diseases: an emerging field of study for diagnostic and therapeutic purposes. Biomed Pharmacother. 2023 Sep;157:114046. doi: https://doi.org/10.1016/j.biopha.2022.114046.
30. Ciregia F, Urbani A, Palmisano G. Extracellular vesicles in brain tumors and neurodegenerative diseases. Front Mol Neurosci. 2017;10:276. doi: https://doi.org/10.3389/fnmol.2017.00276.
31. Mosquera-Heredia MI, Morales LC, Vidal OM, Barceló E, Silvera-Redondo C, Vélez JI, et al. Exosomes: potential disease biomarkers and new therapeutic targets. Biomedicines. 2021 Aug;9(8):1031. doi: https://doi.org/10.3390/biomedicines9081031.
32. Perpiñá-Clérigues C, Mellado S, Català-Senent JF, Ibáñez F, Costa P, Marcos M, et al. Lipidomic landscape of circulating extracellular vesicles isolated from adolescents exposed to ethanol intoxication: a sex difference study. Biol Sex Differ. 2023 Apr;14(1):22. doi: https://doi.org/10.1186/s13293-023-00502-1.
33. Fyfe J, Casari I, Manfredi M, Falasca M. Role of lipid signalling in extracellular vesicles-mediated cell-to-cell communication. Cytokine Growth Factor Rev. 2023;73:20–6. doi: https://doi.org/10.1016/j.cytogfr.2023.03.004.
34. Murphy SA, Nicolaou A. Lipidomics applications in health, disease and nutrition research. Mol Nutr Food Res. 2013 Aug;57(8):1336–46. doi: https://doi.org/10.1002/mnfr.201200790.
35. Gross RW, Han X. Lipidomics at the interface of structure and function in systems biology. Chem Biol. 2011 Mar;18(3):284–91. doi: https://doi.org/10.1016/j.chembiol.2011.02.011.
36. Wenk MR. Lipidomics: new tools and applications. Cell. 2010 Dec;143(6):888–95. doi: https://doi.org/10.1016/j.cell.2010.11.033.
37. Ghadami S, Dellinger K. The lipid composition of extracellular vesicles: applications in diagnostics and therapeutic delivery. Front Mol Biosci. 2023;10:1208161. doi: https://doi.org/10.3389/fmolb.2023.1208161.
38. Watson AD. Thematic review series: systems biology approaches to metabolic and cardiovascular disorders. Lipidomics: a global approach to lipid analysis in biological systems. J Lipid Res. 2006 Oct;47(10):2101–11. doi: https://doi.org/10.1194/jlr.R600002-JLR200.
39. Bou Khalil M, Hou W, Zhou H, Elisma F, Swayne LA, Blanchard AP, et al. Lipidomics era: accomplishments and challenges. Mass Spectrom Rev. 2010 Nov–Dec;29(6):877–929. doi: https://doi.org/10.1002/mas.20294.
40. Wolf C, Quinn PJ. Lipidomics: practical aspects and applications. Prog Lipid Res. 2008 Jan;47(1):15–36. doi: https://doi.org/10.1016/j.plipres.2007.09.001.
41. Massey KA, Nicolaou A. Lipidomics of oxidized polyunsaturated fatty acids. Free Radic Biol Med. 2013 Jun;59:45–55. doi: https://doi.org/10.1016/j.freeradbiomed.2012.08.565.
42. Hulbert AJ, Turner N, Storlien LH, Else PL. Dietary fats and membrane function: implications for metabolism and disease. Biol Rev Camb Philos Soc. 2005 Feb;80(1):155–69. doi: https://doi.org/10.1017/S1464793104006578.
43. Decsi T, Csabi G, Torok K, Erhardt E, Minda H, Burus I, et al. Polyunsaturated fatty acids in plasma lipids of obese children with and without metabolic cardiovascular syndrome. Lipids. 2000 Nov;35(11):1179–84. doi: https://doi.org/10.1007/s11745-000-0626-8.
44. Han X, Gross RW. The foundations and development of lipidomics. J Lipid Res. 2022 Feb;63(2):100164. doi: https://doi.org/10.1016/j.jlr.2021.100164.
45. Han X. Lipidomics for studying metabolism. Nat Rev Endocrinol. 2016 Jul;12(11):668–79. doi: https://doi.org/10.1038/nrendo.2016.98.
46. Ahluwalia K, Ebright B, Chow K, Dave P, Mead A, Poblete R, et al. Lipidomics in understanding pathophysiology and pharmacologic effects in inflammatory diseases: considerations for drug development. Metabolites. 2022 Apr;12(4):323. doi: https://doi.org/10.3390/metabo12040323.
47. Su H, Rustam YH, Masters CL, Makalic E, McLean CA, Hill AF, et al. Characterization of brain-derived extracellular vesicle lipids in Alzheimer's disease. J Extracell Vesicles. 2021 May;10(7):e12089. doi: https://doi.org/10.1002/jev2.12089.
48. de Freitas RCC, Hirata RDC, Hirata MH, Aikawa E. Circulating extracellular vesicles as biomarkers and drug delivery vehicles in cardiovascular diseases. Biomolecules. 2021 Mar;11(3):388. doi: https://doi.org/10.3390/biom11030388.
49. Al Abdullah S, Cocklereece I, Dellinger K. Unlocking the potential of circulating small extracellular vesicles in neurodegenerative disease through targeted biomarkers and advancements in biosensing. Explor BioMat-X. 2024;1(2):100–23. doi: https://doi.org/10.37349/biomatx.2024.00008.
50. Zhou E, Li Y, Wu F, Guo M, Xu J, Wang S, et al. Circulating extracellular vesicles are effective biomarkers for predicting response to cancer therapy. EBioMedicine. 2021 May;67:103365. doi: https://doi.org/10.1016/j.ebiom.2021.103365.
51. Garcia-Contreras M, Thakor AS. Extracellular vesicles in Alzheimer's disease: from pathology to therapeutic approaches. Neural Regen Res. 2023 Jan;18(1):18–22. doi: https://doi.org/10.4103/1673-5374.345471.
52. Khoury S, Canlet C, Lacroix MZ, Berdeaux O, Jouhet J, Bertrand-Michel J. Quantification of lipids: model, reality, and compromise. Biomolecules. 2018 Dec;8(4):174. doi: https://doi.org/10.3390/biom8040174.
53. Saini RK, Prasad P, Shang X, Keum YS. Advances in lipid extraction methods: a review. Int J Mol Sci. 2021 Dec;22(24):13663. doi: https://doi.org/10.3390/ijms222413663.
54. Duché G, Sanderson JM. The chemical reactivity of membrane lipids. Chem Rev. 2024 Mar;124(6):3284–330. doi: https://doi.org/10.1021/acs.chemrev.3c00516.

55. Fam TK, Klymchenko AS, Collot M. Recent advances in fluorescent probes for lipid droplets. Materials (Basel). 2018 Sep;11(9):1768. doi: https://doi.org/10.3390/ma11091768.

56. Stoll LL, Spector AA. Changes in serum influence the fatty acid composition of established cell lines. In Vitro. 1984 Sep;20(9):732–8. doi: https://doi.org/10.1007/BF02618896.

57. Yang K, Han X. Lipidomics: techniques, applications, and outcomes related to biomedical sciences. Trends Biochem Sci. 2016 Nov;41(11):954–69. doi: https://doi.org/10.1016/j.tibs.2016.08.010.

58. Li L, Han J, Wang Z, Liu J, Wei J, Xiong S, et al. Mass spectrometry methodology in lipid analysis. Int J Mol Sci. 2014 Jun;15(6):10492–507. doi: https://doi.org/10.3390/ijms150610492.

59. Skotland T, Sagini K, Sandvig K, Llorente A. An emerging focus on lipids in extracellular vesicles. Adv Drug Deliv Rev. 2020 Aug;159:308–21. doi: https://doi.org/10.1016/j.addr.2020.01.003.

60. Sancho-Albero M, Jarne C, Savirón M, Martín-Duque P, Membrado L, Cebolla VL, et al. High-performance thin-layer chromatography-densitometry-tandem ESI-MS to evaluate phospholipid content in exosomes of cancer cells. Int J Mol Sci. 2022 Jan;23(3):1281. doi: https://doi.org/10.3390/ijms23031281.

61. Liangsupree T, Multia E, Saarinen J, Ruiz-Jimenez J, Kemell M, Riekkola ML. Raman spectroscopy combined with comprehensive gas chromatography for label-free characterization of plasma-derived extracellular vesicle subpopulations. Anal Biochem. 2022 Jun;647:114672. doi: https://doi.org/10.1016/j.ab.2022.114672.

62. Imanbekova M, Suarasan S, Rojalin T, Mizenko RR, Hilt S, Mathur M, et al. Identification of amyloid beta in small extracellular vesicles via Raman spectroscopy. Nanoscale Adv. 2021 Jul;3(14):4119–32. doi: https://doi.org/10.1039/D1NA00300H.

63. Johnson KR, Ellis G, Toothill C. The sulfophosphovanillin reaction for serum lipids: a reappraisal. Clin Chem. 1977 Sep;23(9):1669–78. doi: https://doi.org/10.1093/clinchem/23.9.1669.

64. Ullah MS, Zhivonitko VV, Samoylenko A, Zhyvolozhnyi A, Viitala S, Kankaanpää S, et al. Identification of extracellular nanoparticle subsets by nuclear magnetic resonance. Chem Sci. 2021 Jun;12(24):8311–9. doi: https://doi.org/10.1039/D1SC01313A.

65. Jakubec M, Maple-Grødem J, Akbari S, Nesse S, Halskau Ø, Mork-Jansson AE. Plasma-derived exosome-like vesicles are enriched in lysophospholipids and pass the blood–brain barrier. PLoS One. 2020 Sep;15(9):e0232442. doi: https://doi.org/10.1371/journal.pone.0232442.

66. Bai L, Bu F, Li X, Zhang S, Min L. Mass spectrometry-based extracellular vesicle micromolecule detection in cancer biomarker discovery: an overview of metabolomics and lipidomics. View (Beijing). 2023 Oct;4(5):20220086. doi: https://doi.org/10.1002/viw.20220086.

67. Züllig T, Trötzmüller M, Köfeler HC. Lipidomics from sample preparation to data analysis: a primer. Anal Bioanal Chem. 2020 Apr;412(10):2191–209. doi: https://doi.org/10.1007/s00216-019-02241-7.

68. Huang T, He J. Characterization of extracellular vesicles by size-exclusion high-performance liquid chromatography (HPLC). Methods Mol Biol. 2017;1660:191–9. doi: https://doi.org/10.1007/978-1-4939-7253-1_16.

69. Lam SM, Zhang C, Wang Z, Ni Z, Zhang S, Yang S, et al. A multi-omics investigation of the composition and function of extracellular vesicles along the temporal trajectory of COVID-19. Nat Metab. 2021 Jul;3(7):909–22. doi: https://doi.org/10.1038/s42255-021-00405-6.

70. Peterka O, Jirásko R, Chocholoušková M, Kuchař L, Wolrab D, Hájek R, et al. Lipidomic characterization of exosomes isolated from human plasma using various mass spectrometry techniques. Biochim Biophys Acta Mol Cell Biol Lipids. 2020 May;1865(5):158634. doi: https://doi.org/10.1016/j.bbalip.2020.158634.

71. Aresta AM, De Vietro N, Zambonin C. Analysis and characterization of the extracellular vesicles released in non-cancer diseases using matrix-assisted laser desorption ionization/mass spectrometry. Int J Mol Sci. 2024 Apr;25(8):3952. doi: https://doi.org/10.3390/ijms25083952.

72. Lobasso S, Tanzarella P, Mannavola F, Tucci M, Silvestris F, Felici C, et al. A lipidomic approach to identify potential biomarkers in exosomes from melanoma cells with different metastatic potential. Front Physiol. 2021;12:748895. doi: https://doi.org/10.3389/fphys.2021.748895.

73. Lydic TA, Townsend S, Adda CG, Collins C, Mathivanan S, Reid GE. Rapid and comprehensive ‘shotgun’ lipidome profiling of colorectal cancer cell derived exosomes. Methods. 2015 Oct;87:83–95. doi: https://doi.org/10.1016/j.ymeth.2015.05.014.

74. Byrdwell WC. Atmospheric pressure chemical ionization mass spectrometry for analysis of lipids. Lipids. 2001 Apr;36(4):327–46. doi: https://doi.org/10.1007/s11745-001-0731-6.

75. Wang C, Wang M, Han X. Applications of mass spectrometry for cellular lipid analysis. Mol Biosyst. 2015 Mar;11(3):698–713. doi: https://doi.org/10.1039/C4MB00607B.

76. Dill AL, Ifa DR, Manicke NE, Ouyang Z, Cooks RG. Mass spectrometric imaging of lipids using desorption electrospray ionization. J Chromatogr B Analyt Technol Biomed Life Sci. 2009 Sep;877(26):2883–9. doi: https://doi.org/10.1016/j.jchromb.2009.03.043.

77. Morris CB, Poland JC, May JC, McLean JA. Fundamentals of ion mobility–mass spectrometry for the analysis of biomolecules. In: Paglia G, Astarita G. editors. Ion Mobility–Mass Spectrometry: Methods and Protocols. New York: Springer; 2020. p. 1–31. doi: https://doi.org/10.1007/978-1-0716-0161-1_1.

78. Li M, Yang L, Bai Y, Liu H. Analytical methods in lipidomics and their applications. Anal Chem. 2014 Jan;86(1):161–75. doi: https://doi.org/10.1021/ac403852f.

79. Jia F, Zhao X, Zhao Y. Advancements in ToF-SIMS imaging for life sciences. Front Chem. 2023 Aug;11:1237408. doi: https://doi.org/10.3389/fchem.2023.1237408.

80. Sakamoto T, Koizumi M, Kawasaki J, Yamaguchi J. Development of a high lateral resolution TOF-SIMS apparatus for single particle analysis. Appl Surf Sci. 2008 Nov;255(4):1617–20. doi: https://doi.org/10.1016/j.apsusc.2008.06.166.

81. Graham DJ, Gamble LJ. Back to the basics of time-of-flight secondary ion mass spectrometry data analysis of bio-related samples. II. Data processing and display. Biointerphases. 2023 May;18(3):031004. doi: https://doi.org/10.1116/6.0002917.

82. Yoon S, Lee TG. Biological tissue sample preparation for time-of-flight secondary ion mass spectrometry (ToF–SIMS) imaging. Nano Converg. 2018;5(1):24. doi: https://doi.org/10.1186/s40580-018-0154-5.

83. Marzec ME, Wojtysiak D, Połtowicz K, Nowak J, Pedrys R. Study of cholesterol and vitamin E levels in broiler meat from different feeding regimens by TOF-SIMS. Biointerphases. 2016 Apr;11(2):02A326. doi: https://doi.org/10.1116/1.4944749.

84. Passarelli MK, Winograd N. Lipid imaging with time-of-flight secondary ion mass spectrometry (ToF-SIMS). Biochim Biophys Acta. 2011 Nov;1811(11):976–90. doi: https://doi.org/10.1016/j.bbalip.2011.06.021.

85. McMahon JM, Short RT, McCandlish CA, Brenna JT, Todd PJ. Identification and mapping of phosphocholine in animal tissue by static secondary ion mass spectrometry and tandem mass spectrometry. Rapid Commun Mass Spectrom. 1996 Mar;10(3):335–40. doi: https://doi.org/10.1002/(SICI)1097-0231(19960315)10:3<335::AID-RCM478>3.0.CO;2-B.

86. Seedorf U, Fobker M, Voss R, Meyer K, Kannenberg F, Meschede D, et al. Smith-Lemli-Opitz syndrome diagnosed by using time-of-flight secondary-ion mass spectrometry. Clin Chem. 1995 Apr;41(4):548–52. doi: https://doi.org/10.1093/clinchem/41.4.548.
87. Börner K, Malmberg P, Månsson JE, Nygren H. Molecular imaging of lipids in cells and tissues. Int J Mass Spectrom. 2007 Feb;260(2–3):128–36. doi: https://doi.org/10.1016/j.ijms.2006.09.010.
88. Touboul D, Roy S, Germain DP, Chaminade P, Brunelle A, Laprévote O. MALDI-TOF and cluster-TOF-SIMS imaging of Fabry disease biomarkers. Int J Mass Spectrom. 2007 Feb;260(2–3):158–65. doi: https://doi.org/10.1016/j.ijms.2006.10.007.
89. Pacholski ML, Cannon DM Jr, Ewing AG, Winograd N. Static time-of--flight secondary ion mass spectrometry imaging of freeze-fractured, frozen-hydrated biological membranes. Rapid Commun Mass Spectrom. 1998;12(18):1232–5. doi: https://doi.org/10.1002/(SICI)1097--0231(19980830)12:18<1232::AID-RCM323>3.0.CO;2-J.
90. Nygren H, Börner K, Hagenhoff B, Malmberg P, Månsson JE. Localization of cholesterol, phosphocholine and galactosylceramide in rat cerebellar cortex with imaging TOF-SIMS equipped with a bismuth cluster ion source. Biochim Biophys Acta. 2005 Dec;1737(2–3):102–10. doi: https://doi.org/10.1016/j.bbalip.2005.10.004.
91. Touboul D, Brunelle A, Halgand F, De La Porte S, Laprévote O. Lipid imaging by gold cluster time-of-flight secondary ion mass spectrometry: application to Duchenne muscular dystrophy. J Lipid Res. 2005 Jul;46(7):1388–95. doi: https://doi.org/10.1194/jlr.M400473-JLR200.
92. Prinz C, Hook F, Malm J, Sjövall P. Structural effects in the analysis of supported lipid bilayers by time-of-flight secondary ion mass spectrometry. Langmuir. 2007 Jul;23(15):8035–41. doi: https://doi.org/10.1021/la700803m.
93. Ostrowski SG, Szakal C, Kozole J, Roddy TP, Xu J, Ewing AG, et al. Secondary ion MS imaging of lipids in picoliter vials with a buckminsterfullerene ion source. Anal Chem. 2005 Oct;77(19):6190–6. doi: https://doi.org/10.1021/ac050828p.
94. Touboul D, Kollmer F, Niehuis E, Brunelle A, Laprévote O. Improvement of biological time-of-flight secondary ion mass spectrometry imaging with a bismuth cluster ion source. J Am Soc Mass Spectrom. 2005 Oct;16(10):1608–18. doi: https://doi.org/10.1016/j.jasms.2005.04.032.
95. Monroe EB, Jurchen JC, Lee J, Rubakhin SS, Sweedler JV. Vitamin E imaging and localization in the neuronal membrane. J Am Chem Soc. 2005 Sep;127(35):12152–3. doi: https://doi.org/10.1021/ja053576v.
96. Amemiya T, Tozu M, Ohashi Y. Time-of-flight secondary ion mass spectrometry can replace histochemistry demonstration of fatty acids in the retina. Jpn J Ophthalmol. 2004 May;48(3):287–93. doi: https://doi.org/10.1016/j.jjo.2004.02.001.
97. Song Z, Wang Z, Zhao H, Cai L, Li Z, Zhang S, et al. Metabolic fingerprinting of cell types in mouse skeletal muscle by combining TOF-SIMS with immunofluorescence staining. Analyst. 2020 Nov;145(21):6901–9. doi: https://doi.org/10.1039/D0AN01226A.
98. Marzec ME, Wojtysiak D, Połtowicz K, Nowak J. ToF-SIMS spectrometry to observe fatty acid profiles of breast tissues in broiler chicken subjected to varied vegetable oil diet. J Mass Spectrom. 2020 Mar;55(3):e4476. doi: https://doi.org/10.1002/jms.4476.
99. Ren J, Li HW, Chen L, Zhang M, Liu YX, Zhang BW, et al. Mass spectrometry imaging-based single-cell lipidomics profiles metabolic signatures of heart failure. Research (Wash D C). 2023 Jan;6:0048. doi: https://doi.org/10.34133/research.0048.
100. Jia F, Zhao X, Zhao Y. Advancements in ToF-SIMS imaging for life sciences. Front Chem. 2023 Aug;11:1237408. doi: https://doi.org/10.3389/fchem.2023.1237408.
101. Kim Y, Shon HK, Shin SK, Lee TG. Probing nanoparticles and nanoparticle-conjugated biomolecules using time-of-flight secondary ion mass spectrometry. Mass Spectrom Rev. 2015 Apr;34(2):237–47. doi: https://doi.org/10.1002/mas.21416.
102. Wu K, Jia F, Zheng W, Luo Q, Zhao Y, Wang F. Visualization of metallodrugs in single cells by secondary ion mass spectrometry imaging. J Biol Inorg Chem. 2017 Jul;22(5):653–61. doi: https://doi.org/10.1007/s00775-017-1442-8.
103. Marzec ME, Rząca C, Moskal P, Stępień EŁ. Study of the influence of hyperglycemia on the abundance of amino acids, fatty acids, and selected lipids in extracellular vesicles using ToF-SIMS. Biochem Biophys Res Commun. 2022 Sep;622:30–6. doi: https://doi.org/10.1016/j.bbrc.2022.06.076.
104. Chetwynd AJ, Lynch I. The rise of the nanomaterial metabolite corona, and emergence of the complete corona. Environ Sci Nano. 2020 Apr;7(4):1041–60. doi: https://doi.org/10.1039/C9EN00993J.
105. González-García LE, MacGregor MN, Visalakshan RM, Lazarian A, Cavallaro AA, Morsbach S, et al. Nanoparticles surface chemistry influence on protein corona composition and inflammatory responses. Nanomaterials (Basel). 2022 Feb;12(4):682. doi: https://doi.org/10.3390/nano12040682.
106. Graham DJ, Wilson JT, Lai JJ, Stayton PS, Castner DG. Three-dimensional localization of polymer nanoparticles in cells using ToF--SIMS. Biointerphases. 2016 Jun;11(2):02A301. doi: https://doi.org/10.1116/1.4943168.
107. Belu AM, Davies MC, Newton JM, Patel N. TOF-SIMS characterization and imaging of controlled-release drug delivery systems. Anal Chem. 2000 Nov;72(22):5625–38. doi: https://doi.org/10.1021/ac000450+.