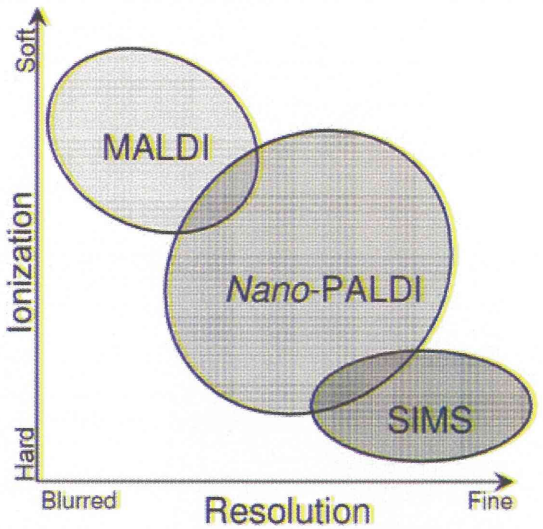

Fig. 6. Applicable areas of matrix-assisted laser desorption/ionization (MALDI), nanoparticle-assisted laser desorption/ionization (nano-PALDI) and secondary ion mass spectrometry (SIMS) techniques in IMS. Three different IMS techniques are compared based on the resolution and the severity of ionization.

and amino groups were linked, resulting in improved ionization efficiency for the small molecules. The improved ionization efficiency presumably results from the capture of analyte molecules close to the fNP surface, which facilitates efficient transfer of laser energy from the NP to the analyte molecules (Fig. 8h). Figure 7 shows representative mass spectra of mouse brain sections obtained using fNP and DHB as respective matrices. Comparison of the spectra shows that in the mass range of $700 < m/z < 900$, signals derived from phospholipids and glycolipids were detected at almost the same intensity using either technique. On the other hand, in the lower mass range, i.e., $100 < m/z < 500$, a larger number of mass peaks with higher intensities were detected using fNP as matrix, whereas when DHB was used as a matrix, most of the intense peaks detected in the same mass region were derived from DHB-originated ions. This example clearly demonstrates the highly effective nature of nano-PALDI for small molecule imaging.

Another important advantage: spraying fNP on the tissue surface did not alter the optical image of the biological tissue surface (Fig. 8). In contrast, when the mouse brain sections were sprayed with a DHB solution, non-homogeneous DHB crystals were formed on the section, which obscured the optical view of the sample surface (Fig. 8a–b). This obscurity resulting

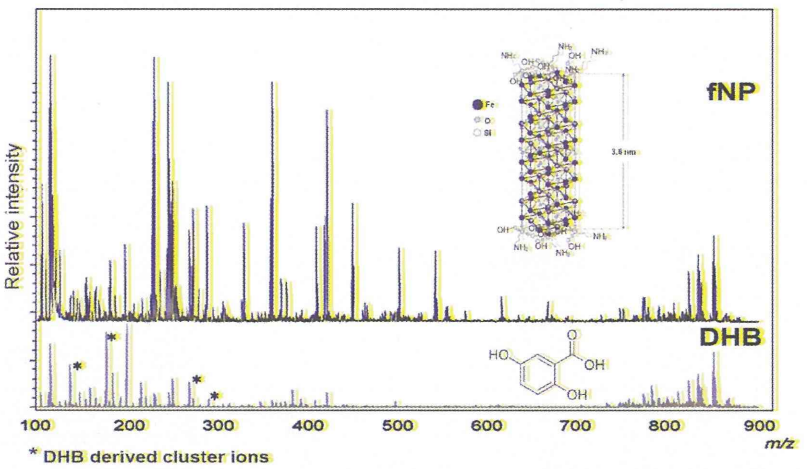

Fig. 7. Representative mass spectra of mouse brain section obtained using functional nanoparticle (fNP) and DHB as respective matrices. Either fNP or 2,5-dihydroxybenzoic acid (DHB) was applied as a matrix on a mouse brain section, and mass spectra were obtained from each applied spot. These mass spectra clearly demonstrate the elimination of DHB-derived background signals as well as increased detection sensitivity for small molecules within the 100 < *m/z* < 600 range using fNP as a matrix. Reprinted from Sahashi et al. 2010 with the permission of Elsevier, Ltd.

from crystal formation with DHB makes it difficult to predefine the tissue region of interest before conducting MS or IMS measurements. Owing to the quite large number of MS measurements performed during the IMS experiment, which is equal to the number of pixels of the resulting image, there is a practical requirement that the measurement area be limited (generally, ten thousand MS measurements per single IMS analysis is the upper limit mainly because of the huge size of the data set). In addition, SEM observation of the DHB coated tissue surface revealed inhomogeneous needle like crystals having typical lengths of > 50 μm (Fig. 8c), which limits the spatial resolution of MALDI-IMS, as described above. In contrast, because of the extremely small particle size of fNPs, spraying these fNPs onto the tissue surface did not alter the optical image of the tissue structure (Fig. 7d–e), thereby allowing the researcher to perform MS and IMS measurements with concomitant optical observation of the tissue structure (using a CCD camera with which MALDI-MS instruments are generally equipped). The SEM images demonstrate that the fNPs which were sprayed onto the tissue surface were distributed in a manner similar to the as-synthesized particles (Fig. 8f–g).

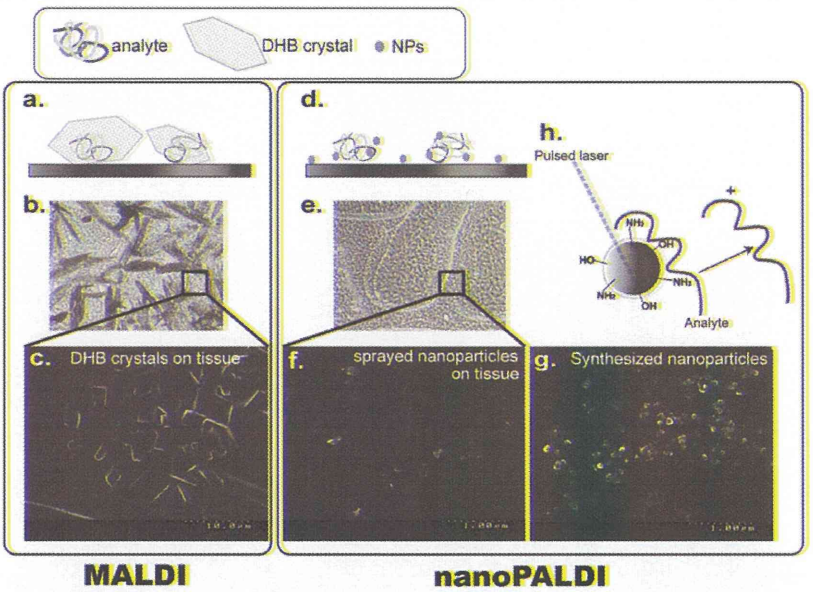

Fig. 8. Principle of nanoparticle-assisted laser desorption/ionization (nano-PALDI) and matrix-assisted laser desorption/ionization based imaging mass spectrometry (MALDI-IMS). Shown are schematic, light microscopic, and scanning electron microscopy images of 2,5-dihydroxybenzoic acid (DHB)/nanoparticles (NPs) applied to biological tissue section. MALDI requires formation of analyte-matrix co-crystals with typical sizes of <50 μm on the tissue section, which limits the spatial resolution of MALDI-IMS (left), whereas in nano-PALDI, the use of NPs as the ionization enhancing reagent eliminates formation of such crystals (right) and enables clear observation of tissue surface even during mass spectrometry measurement. Modified from Taira et al. 2008 with the permission of ACS Publications.

The considerable advantage of nano-PALDI-based IMS in the low *m/z* region is clearly demonstrated in Fig. 9, which shows the results of the feasibility study using IMS measurements performed with both nano-PALDI and MALDI. Tissue samples were obtained from rat cerebella, and NP fluid was sprayed on a thin tissue section of the cerebellum (Fig. 9b, d, f), whereas successive tissue sections were treated by application of DHB (Fig. 9c, e, g). Each IMS measurement was performed at spatial resolution of 15 μm, using a MALDI-TOF/TOF-type instrument. Consistent with the aforementioned features of fNPs, the comparative analysis showed that the use of fNP produced a unique ion distribution image that could not be detected when DHB was used (Fig. 9e and g), and a much finer ion distribution image (Fig. 9d and f), without background noise. On the other hand, ion images with DHB showed crystal-shaped ion localization patterns (especially in Fig. 9g), suggesting that analyte molecules could

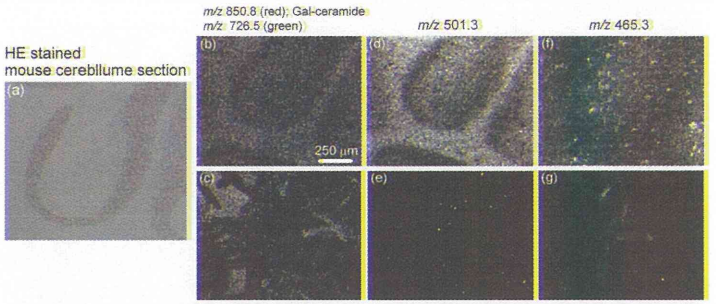

Fig. 9. Nanoparticle-assisted laser desorption/ionization based imaging mass spectrometry (nano-PALDI-IMS) of low molecular weight compounds showing improved ion distribution image quality. Optical images of rat cerebellum tissue before spraying with nanoparticles (NPs) (a) and 2,5-dihydroxybenzoic acid (DHB) solution, and ion images obtained with NPs (b, d, f) and DHB (c, e, g) are shown. Visualized ions were identified as galactosylceramide (C24h:0) and phosphotidylcholine (PC) (diacyl-34:2) by tandem mass spectrometry on both DHB and NP coated sections. Reprinted from Taira et al. 2008 with the permission of ACS Publications.

Color image of this figure appears in the color plate section at the end of the book.

only be ionized from the analyte-matrix co-crystals. Even though the results are only representative, this example clearly demonstrates that the organic matrix crystals severely limit the IMS spatial resolution.

Analysis of Molecular Distribution of Sulfatide

As frequently described, lipids constitute half of the dry weight in the brain, and play important roles especially as fundamental building components of cell structures and as signaling molecules with strong bioactivity (Bosio et al. 1998). Owing to the technical difficulty presented by the insolubility of lipids in aqueous media, the study of lipids has been mainly performed using traditional biochemical techniques. The emergence of IMS as a tool for the imaging of lipids has had significant impact, because there was previously no established technique for two-dimensional mapping of lipids, whereas transcripts can be visualized with oligonucleotide probes using *in situ* hybridization and proteins can be visualized using immunohistochemistry with appropriate antibodies. In this context, lipid imaging by IMS, particularly utilizing nano-PALDI based IMS should prove critical to the interpretation of the role of lipids in brain research.

Nano-PALDI-IMS has also been applied to the mapping of sulfatide distribution. Sulfatides are important lipid components of the myelin sheath. A direct correlation between sulfatide deficiency and neurological disorders, such as Alzheimer's disease (Han et al. 2002) has been reported. Furthermore, one decomposition pathway of sulfatides is catalyzed by

arylsulfatase A (ASA) and the functional deficiency of ASA results in metachromatic leukodystrophy (MLD), which causes the accumulation of sulfatide in lysozymal storage deposits, and eventually, demyelination in the peripheral and central nervous systems (PNS and CNS) (Krivit 2004). In addition, structural variations of sulfatide arise from hydroxylation of the fatty acid moiety as shown by the arrow in Fig. 10. The hydroxylation is catalyzed by fatty acid 2-hydroxylase (FA2H), which also causes leukodystrophy with spastic paraparesis and dystonia (Kruer et al. 2010).

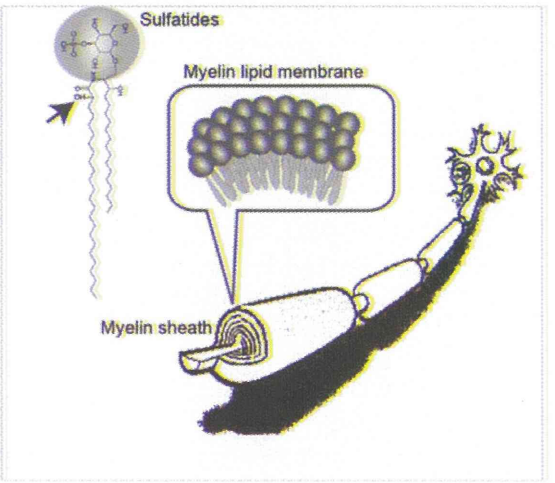

Fig. 10. Schematic representation of myelin sheath and one of its major lipid components, sulfatides. Nanoparticle-assisted laser desorption/ionization imaging mass spectrometry (nano-PALDI-IMS) was applied to the mapping of sulfatide distribution. Sulfatides are important lipid components of the myelin sheath. The black arrow indicates the hydroxylation site on the fatty acid moiety that causes structural variation.

The distribution pattern of sulfatide in the brain has been assessed by using the anti-sulfatide antibody to track the distribution of sulfatide in the CNS and PNS of rodent brains (Pernber et al. 2002). However, immunostaining with this antibody did not allow for discrimination of the fatty acid moiety of this lipid specie or of the presence/absence of the abovementioned hydroxylation. In comparison, based on the MS detection principle, distinct images of these species were obtained using IMS (Ageta et al. 2009). Figure 11 shows different regions of the dentate gyrus of rat hippocampus, such as the granular cell layer (GCL), inner molecular layer (IML), and middle molecular layer (MML) (left column), as well as the intensity of the different mass peaks in these regions (right column). It was found that the intensity of the peaks with m/z of 906.3,

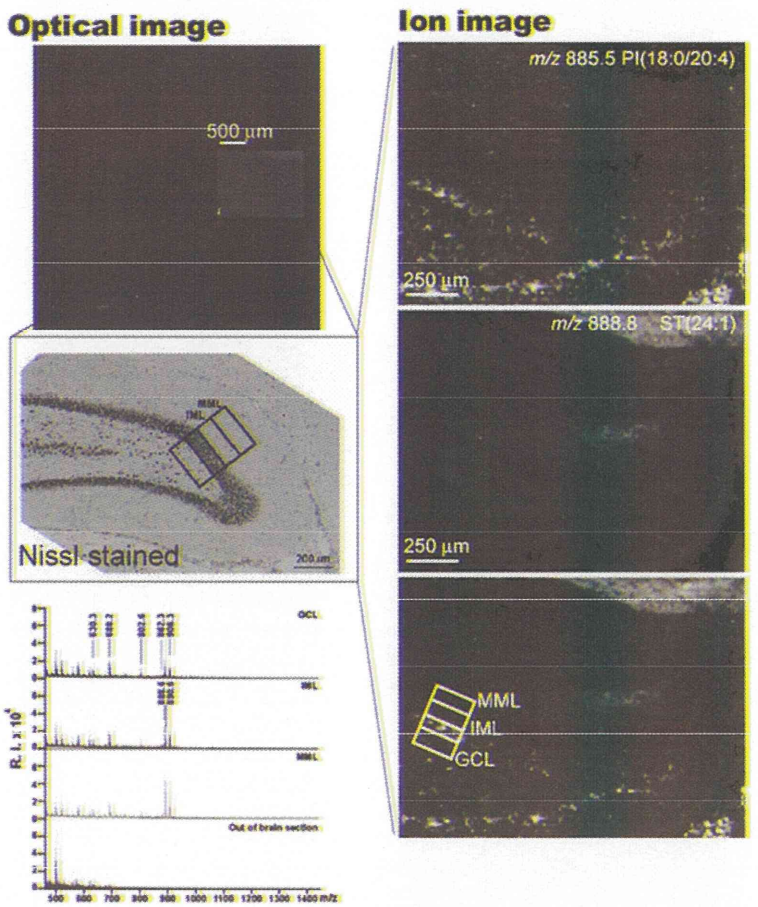

Fig. 11. Nanoparticle-assisted laser desorption/ionization improves spatial resolution in imaging mass spectrometry (IMS). Optical image of rat hippocampus indicating measurement area for nanoparticle-based IMS is shown along with Nissl-stained section indicating fine layer structure of rat hippocampus (left panels). Ion images, which reveal hippocampal layer specific distribution of phosphatidylinositol (18:0/20:4) at *m/z* 885.5 and sulfatide (24:1) at *m/z* 888.8, are presented (right panels). GCL = granular cell layer, IML = inner molecular layers, and MML = middle molecular layers. Reprinted from Ageta et al. 2009 (left column) and Sugiura and Setou 2010b (right column) with the permission of Springer.

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904.6, 890.6, and 888.6 was higher in the MML region than in the GCL and IML regions, whereas the mass peaks with *m/z* of 862.3, 802.6, 688.2, and 630.3 were detected in the GCL, IML, and MML regions only, indicating their biological origin. Therefore, this technique can potentially be used to explore physiological processes for better understanding of diagnostics and the pathology of neurological disorders, such as leukodystrophy and Alzheimer's disease (Ageta et al. 2009).

Key Facts

- A key fact about MALDI-imaging mass spectrometry (IMS) is that it is an MS-based molecular imaging technique with the following unique advantages. (1) MALDI-IMS can be used for the visualization of the distribution of large numbers of biomolecules in cells and tissues, ranging from small metabolite molecules to much larger proteins. (2) MALDI-IMS does not require any specific chemical labels or probes. (3) MALDI-IMS is a "non-targeted" imaging method. (4) MALDI-IMS enables the simultaneous imaging of multiple types of molecular species.
- A key fact about nanoparticle (NP)-assisted laser desorption/ionization (nano-PALDI)-based IMS is that it is an IMS technique, in which the organic matrix is replaced with NPs such as gold, silver, and titanium. Recently, researchers have begun using NPs for MS research owing to the progress in the development of NP technology, which has made various kinds of NPs available. In MS research, the use of NPs has been studied particularly in the analyses of small molecules, because of the following advantages. (1) The use of nano-PALDI may eliminate background noise generated by organic matrix compounds. (2) Judicious choice of NPs allows for the efficient analysis of analyte molecules that are otherwise difficult to ionize using conventional organic matrices.

Summary

- Imaging mass spectrometry (IMS) enables visualization of the distribution of various biomolecules in biological tissue sections.
- IMS has several unique advantages—(1) It does not require any specific chemical labels or probes. (2) It is a "non-targeted" imaging method. (3) The simultaneous imaging of multiple types of molecular species is available.
- MALDI-IMS in particular, which is a soft ionization method using an organic "matrix," can be used for a large number of biomolecules ranging from small metabolite molecules to much larger proteins.

- Although MALDI-based IMS has promising capacity for the imaging of small metabolites, this technique has a critical problem in spatial resolution derived from matrix crystallization.
- In nanoparticle (NP)-assisted laser desorption/ionization (nano-PALDI)-based IMS, the organic matrix is replaced with NPs, and therefore, the matrix crystallization process is eliminated; therefore, it can be used to overcome the resolution problems of MALDI-IMS.
- Recent development of NPs with different core metals, surface modifications, and particle diameters has expanded the measurable range of analytes as well as the application of the analyses to physiological processes and the diagnosis and pathophysiology of complex biological process, especially in the brain.

Abbreviations

ASA	:	Arylsulfatase A
AuNP	:	Gold Nanoparticle
AgNP	:	Silver Nanoparticle
CCD	:	Charge-Coupled Device
CHCA	:	α-Cyano-4-Hydroxycinnamic Acid
CNS	:	Central Nervous System
DHB	:	Dihydroxy Benzoic Acid
DNA	:	Deoxyribonucleic Acid
ESI	:	Electrospray Ionization
FA2H	:	Fatty Acid 2-Hydroxylase
fNP	:	Functional Nanoparticle
GCL	:	Granular Cell Layer
HE	:	Hematoxilin-Eosin
IML	:	Inner Molecular Layer
IMS	:	Imaging Mass Spectrometry
ITO	:	Indium Tin Oxide
MALDI	:	Matrix-Assisted Laser Desorption/Ionization
MLD	:	Metachromatic Leukodystrophy
MML	:	Middle Molecular Layer
MS	:	Mass Spectrometry
m/z	:	Mass-to-Charge Ratio
nano-PALDI	:	Nanoparticle-Assisted Laser Desorption/Ionization
NP	:	Nanoparticle
NSCLC	:	Non-Small Cell Lung Cancer
PC	:	Phosphotidylcholine
PNS	:	Peripheral Nervous System
SEM	:	Scanning Electron Microscopy
SIMS	:	Secondary Ion Mass Spectrometry

SiO_2	:	Silicon dioxide
siRNA	:	Short-Interfering Ribonucleic Acid
TiO_2	:	Titanium Dioxide
TOF	:	Time-of-Flight
WBA	:	Whole-Body Autoradiography

Key Terms

- MS (Mass spectrometry): Analytical technique that measures the mass (m)-to-charge (z) ratio of charged atoms, molecules, and molecular clusters/fragments.
- IMS (Imaging MS): Molecular imaging technique based on MS.
- MALDI (Matrix-assisted laser desorption/ionization): A soft ionization method used for MS using an analyte ionization-enhancing reagent, called a matrix, which allows the analysis of biomolecules and large organic molecules.
- NP (nanoparticle): A particle whose diameter is 100~1000 nm.
- nano-PALDI (NP-assisted laser desorption/ionization): An ionization method used for MS using NP as an analyte ionization-enhancing reagent.

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