# Chapter 1
# IMS as an Historical Innovation

**Mitsutoshi Setou**

**Abstract** The development of mass spectrometry has recently entered a new phase. The previous limitation of mass analyses of biomolecules confined the imagination of researchers to a single location from which samples were gathered; however, an innovation in mass spectrometry has now enabled the provision of additional two-dimensional (2D) axes of recognition on tissue sections. The innovatory technology – imaging mass spectrometry – can visualize the distribution of molecules. It has attracted a great deal of attention in the analyses of drug effects, in screening of drugs, and in support for medical diagnoses. In this chapter, we describe imaging mass spectrometry as an historical innovation and the significance of publishing this protocol book. I strongly believe that this book provides a critical basis for future studies on imaging mass spectrometry.

Imaging mass spectrometry (IMS) is an imaging technique based on mass spectrometry. Images are reconstructed from the mass spectrum charts from thousands of spots from biological tissues to show the distribution of various molecules. The ionization techniques often used are MALDI (matrix-assisted laser desorption ionization), DESI (desorption electrospray ionization), or SIMS (secondary ion mass spectrometry) [1].

SIMS imaging, which uses the second electronic ion, was theoretically invented in 1949 by Herzog and Viehb of Vienna University. The first SIMS device was completed by Liebel and Herzog in 1961, and it was utilized for the surface analysis of metals, which is its widest use at present. The high resolution obtained in this imaging method was comparable to that required for microscopic levels; however, it is not suitable for analyses of biological macromolecules

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M. Setou (ed.), *Imaging Mass Spectrometry*: *Protocols for Mass Microscopy*,
DOI 10.1007/978-4-431-09425-8_1, © Springer 2010

because the second electronic ion beam breaks the structure. In 1997, the group of Dr. Caprioli of Vanderbilt University presented the first paper regarding MALDI imaging [2]. DESI imaging is a relatively new technique, first reported by the group of Dr. Cooks [3]. Dr. Heeren originally reported the stigmatic type of IMS instrument [4]. Recently, our group first developed high-resolution IMS (mass microscopy), such as nanoparticle-assisted laser desorption/ionization (nano-PALDI) [5], for microscopic observation.

The major applications of IMS technology are in a pharmaceutical company, such as for pharmacokinetic monitoring, pharmacotoxicology, and pharmacometabolomes (Fig. 1.1). Although the image resolution of in vivo ADME (absorption, distribution, metabolism, and excretion) by current IMS technology is relatively lower than that obtained using isotope-labeled compounds, preparing for IMS-based experimentation is simpler and easier than for other methods, particularly when thousands of lead compounds need to be tested. IMS, with its higher image resolution, will contribute greatly to understanding of drug action mechanisms, and so they are expected to improve in the near future. Further, IMS is a strong and perhaps unique tool for performing so-called metabolome-mapping while using only a single-sample analysis, thus monitoring the metabolic behavior of thousands of molecules in terms of their quantitative and positional properties.

With the advantage of no labeling, IMS has opened a new frontier in medical and clinical applications. Other molecular imaging techniques such as green fluorescent protein (GFP) labeling or immunohistochemistry require labeling. Lipids and low molecular weight compounds in tissue sections cannot be observed with those conventional microscopic and electron microscopic techniques; therefore, no distribution map of these molecules in tissue structure has to date been described in the scientific literature or medical textbooks. IMS is making possible a characteristic distribution map of lipids (Fig. 1.2), thus also making a major impact on lipid research.

Ordinary techniques for proteomic and metabolomic analysis cannot be applied to biopsies because most of the samples are extremely small to minimize patient burden. For example, if you have a 1-$mm^3$ sample of biopsy tissue, 3 μl buffer solution

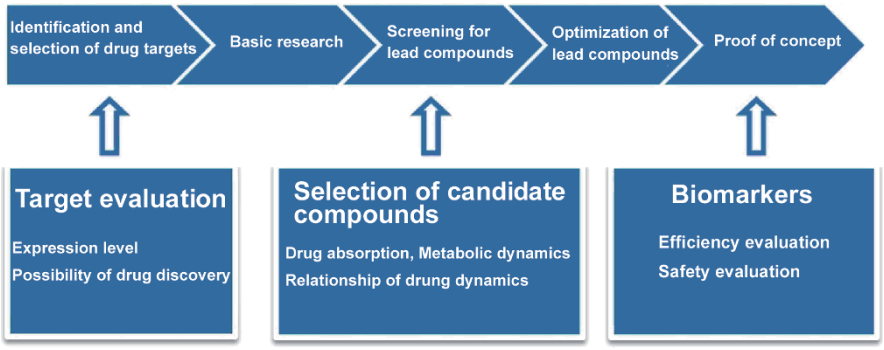


**Fig. 1.1** Processes of new drug development and areas of application of mass microscopy

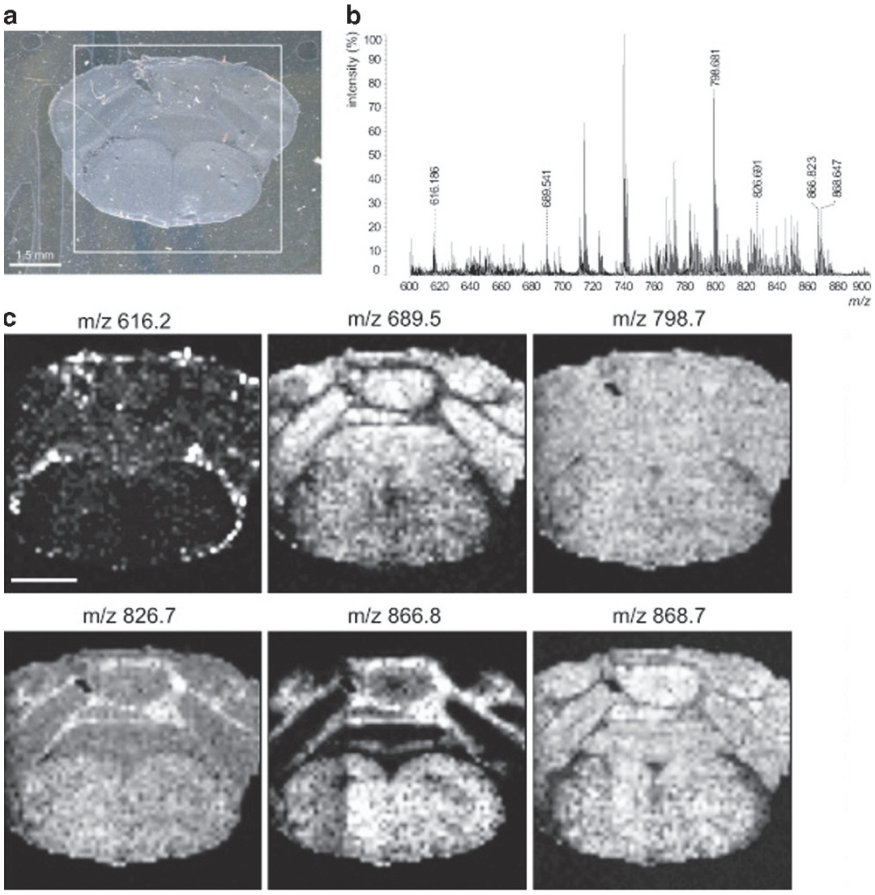


**Fig. 1.2** Characteristic lipid distribution in a rodent brain (Reprinted from Shimma et al., Anal Chem 80(3):878–885.)

is used to dissolve it. Such minute amounts of sample solution would be lost by nonspecific adsorption during subsequent preparation by centrifugation and chromatography. In contrast, when using the IMS technique, the sample would first be flash-frozen, and several μm-thick tissue sections would then be obtained to perform an accurate metabolomic analysis [6, 7]. IMS is expected to become a standard method for reviewing clinical metabolomes because its technique is simple and easy. The mass microscope is now standing alongside computed tomography (CT), positron emission tomography (PET), and magnetic resonance imaging (MRI) in our hospital at Hamamatsu University School of Medicine (Table 1.1).

Any innovation is based on previous efforts. Isaac Newton refers to these achievements as "standing on the shoulders of giants." Within various great innovations, several developments sometimes appear to embody a "great leap" from older discoveries and newborn innovations. Figure 1.3 is my personal view of innovations

**Table 1.1** Comparison of characteristics of molecular visualization methods

| | MRI | NIRF | PET | Biolu. | WBAL | Opt. | IMS |
|---|---|---|---|---|---|---|---|
| In vivo | ✓ | ✓ | ✓ | ✓ | ✗ | ✗ | ✗ |
| Sensitivity | μM | nM | pM | nM | nM | nM | μM |
| Resolution | 50 μm | 5 mm | 5 mm | 1 mm | 10 μm | 1 μm | 50 μm |
| Time required | min | min | min | s | day | min | min |
| Labeling | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Dimension | 3D | 2(3)D | 3D | 2(3)D | 2D | 2D | 2D |
| Cost | ¥¥¥ | ¥ | ¥¥¥¥ | ¥ | ¥¥ | ¥ | ¥¥ |

*MRI* magnetic resonance imaging, *NIRF* near-infrared fluorescence imaging, *PET* positron emission tomography, *Biolu.* bioluminescence, *WBAL* whole-body autoradioluminography, *Opt.* optical, *IMS* imaging mass spectrometry

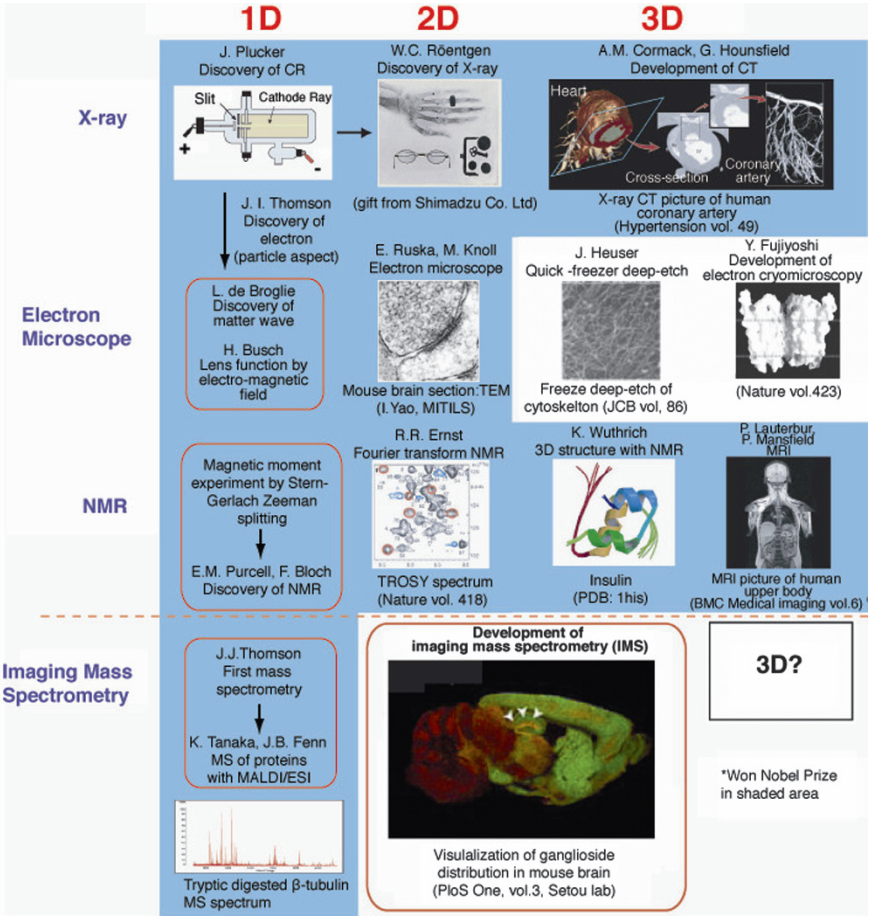


**Fig. 1.3** Historical view of the development of analytical instruments. The progress of imaging technology has been categorized by its technology (*longitudinal axis*) and its analytical dimensions (*horizontal axis*). *NMR* nuclear magnetic resonance. Work shown with *blue area* has received a Nobel Prize. *CR* cathode ray, *NMR* nuclear magnetic resonance, *CT* computed tomography, *TEM* transmission electron microscopy

made in modern biotechnology. The "ground-breaking steps" taken en route to generating innovation – "quantum leaps" – emerge and demonstrate certain steps in the dimensions of analysis. Cathode-ray equipment, for example, evolved into X-ray photographs and, in turn, into CT scans. The electron beam has been used in lieu of an electron microscope. Zeeman splitting has been applied to two-dimensional (2D) nuclear magnetic resonance (NMR) images and then to magnetic resonance (MR) images. The steps taken in the process of innovation match the concomitant increases to analytical dimensionality. The first dimension of analysis is based on basic physics. In the second dimension, an innovation is further developed by means of contributions made by the field of chemistry; subsequently, it is applied to biology and/or medical procedures. Obviously, the next breakthrough for the IMS is to make it 3D (three-dimensional). Toward this direction, we recently have developed atmosphere pressure mass microscopy.

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