International Journal of Mass Spectrometry 307 (2011) 112–122

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Imaging of elements and molecules in biological tissues and cells in the low-micrometer and nanometer range

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ARTICLE INFO

Article history:
Received 10 November 2010
Received in revised form 14 January 2011
Accepted 18 January 2011
Available online 13 February 2011

Keywords:
Analytical technique
Biological tissue and cell
High spatial resolution
Nano-scale
Nano-scale laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS)

ABSTRACT

Investigation of small areas of biological tissues or single cells is of particular interest in the life sciences. Chemical imaging in such samples is able to provide the spatial distribution as well as concentrations of elements and molecules present in the sample. At present, the analytical techniques supporting chemical imaging are under intensive development with respect to higher spatial resolution and higher sensitivity and accuracy. In this review, we will focus on the state of the art of advanced mass spectrometric techniques such as secondary ionization mass spectrometry (SIMS), imaging matrix-assisted laser desorption/ionization mass spectrometry (imaging MALDI-MS), nano-scale laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) versus non-mass spectrometric techniques, for instance, synchrotron-based X-ray fluorescence and scanning near-field optical microscopy (SNOM) assist Raman spectroscopy, with lateral resolution down to low-micrometer and nanometer scales. The outstanding features and drawbacks of each technique are also discussed regarding their application on the study of biological samples. The promising future of imaging mass spectrometric techniques, especially nano-scale LA-ICP-MS, for application in biochemical studies with high spatial resolution down to the nanometer range is also discussed.

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1. Introduction

The distribution and local chemical environment of metals and non-metals in tissues and cells is the most fundamental knowledge of any kind of organism. To obtain information concerning the chemical species, as well as their concentrations and locations is crucial for understanding their biological functions and metabolic process. Traditional solution-based analytical methods require the biological samples to be pre-processed by either digestion or desorption in certain materials for chemical degradation. However, the sample preparation steps may disturb the structure and *in vivo* nature of the tissues and cells. In addition, foreign contamination may occur resulting in difficulties in interpreting of the analytical data. Therefore, there is a need to acquire the chemical information directly from the original biological samples. Bioimaging of elements and molecules in tissues and cells thus becomes necessary for this purpose.

In recent years, there has been a growing interest in the development of analytical techniques for chemical bioimaging, and, indeed, different analytical approaches have been established for directly imaging chemicals in biological samples. These techniques include mass spectrometric methods such as laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) [1–4], secondary ionization mass spectrometry (SIMS) [5–8], imaging matrix-assisted laser desorption/ionization mass spectrometry (imaging MALDI-MS) [9–11], synchrotron-based X-ray fluorescence spectroscopy (SR-XRF) [12–15] and synchrotron-based X-ray absorption spectroscopy (SR-XAS) [16–18] (as well as XRF from laboratory X-ray source with relatively lower energy), microscopy techniques based on vibration spectroscopy, for example infrared spectroscopy [19,20] and Raman spectroscopy [21,22], probe-specific detection methods such as confocal fluorescence imaging (CF) via the measurement of fluorescence-labeled molecules [23,24], as well as some hyphenated techniques like scanning or transmission electron microscopy with energy-dispersive X-ray analysis (SEM–EDX or TEM–EDX) [25–27], and so on. Table 1 summarizes the main characteristics of the major bioimaging techniques mentioned above to compare their performances in different applications. Specifically, Fig. 1 gives an overview of the ion sources in mass spectrometric techniques currently used for chemical imaging in the field of life sciences.

In the case of chemical analysis in smaller-size samples such as single cells, sensitive analytical techniques with much higher spatial resolution down to the low-micrometer and even nanometer ranges are required. One strategy is to decrease the size of the

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doi:10.1016/j.ijms.2011.01.019

Table 1
Comparison of chemical imaging techniques for biological tissues and cells in low-μm and -nm ranges illustrated in this review (a more detailed comparison or characteristics of other techniques can be found in Refs. [7,28]).

Technique	Light source	Detection	Vacuum requirement	Sensitivity	Lateral resolution	Information obtained	Limitation	Examples of application in reference
SIMS	Primary ions	Secondary ions	Yes	>ng g^{-1}	10 μm–50 nm (LMIG: <50 nm)	Element and molecular (1–200 Da)	Strong matrix effects, difficult to quantify	[35,38]
Imaging MALDI-MS	Laser	Pseudomolecular ions	Yes	μg g^{-1}	150–20 μm (7 μm in Ref. [46])	Molecular (up to 100 kDa)	Suitable matrix	[52,53]
NF-LA-ICP-MS	Laser	Neutrals post-ionized in ICP	No	>ng g^{-1}	Low-μm down to ~200 nm	Element and isotope (6–250 Da)	Commercial instrument not available	[70–73]
LMD-ICP-MS	Laser	Neutrals post-ionized in ICP	No	μg g^{-1}	Low-μm down to ~300 nm	Element and isotope (6–250 Da)	Laser power	[75]
Aperture SNOM-LA-ToF-MS	Laser	Ionized molecules	No	μg g^{-1}	Low-μm down to ~300 nm	Molecular	Super sensitive MS required for tiny amount of material ablated	[65,67]
Aperture SNOM-Raman	Laser	Raman scattering	No	μg g^{-1}	~100 nm	Molecular	Weak Raman scattering	[106,107]
TERS	Laser	Raman scattering	No	μg g^{-1}	~100 nm	Molecular	Heating of the tip and the sample	[119,120]
μ-XRF	X-ray from SR	X-ray fluorescence	No	0.1 μg g^{-1}	25 μm–50 nm	Element	Access to SR beamline	[88–91]
μ-XAS (XANES)	X-ray from SR	X-ray fluorescence	No	>μg g^{-1}	<100 μm	Oxidation state	Access to SR beamline, references for complex bio-samples	[96,97]

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