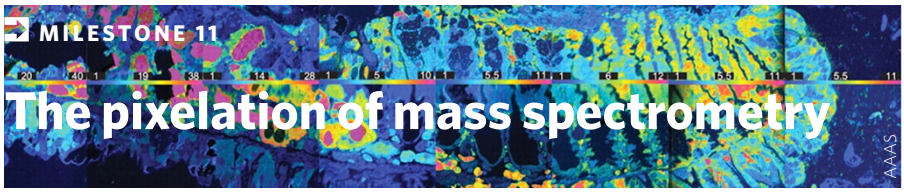

IMS analysis of nitrogen fixation by bacterial symbionts within a marine bivalve. Reproduced from Lechene, C.P., Luyten, Y., McMahon, G. & Distel, D.L., *Science* **317**, 1563–1566, 2007, with permission from AAAS.

The idea of employing mass spectrometry for imaging purposes (imaging mass spectrometry; IMS) was first introduced by Raymond Castaing and Georges Slodzian in 1962, using the principles of secondary ion mass spectrometry (SIMS; see **Milestone 2**). Castaing had begun his career as a research engineer at the Office National d'Etudes et de Recherches Aéronautiques (ONERA), and from then on had nurtured an interest in metal alloys and how their composition varies across a sample.

In SIMS, a sample is bombarded with high-energy 'primary ions', such as Cs^+, O, Ar^+, Ga^+ and In^+, causing an ejection of 'secondary ions' from the surface. These secondary ions are representative of the atomic composition of the surface and can be collected and separated according to their mass ratios. Castaing and Slodzian reasoned that these ions could also be used to obtain information about the specific element or isotopes that are present at a particular position on a metal-alloy surface. They recognized that by analyzing different, defined points across the sample, they could build up an image of which element or isotope was present. Each sampling point would reflect what we now describe as a pixel in an image.

Castaing and Slodzian compared their new SIMS-based approach with an approach based on X-ray emission spectroscopy called 'scanning microanalysis', which Castaing had developed while working with André Guinier in the 1940s and 1950s. Castaing and Slodzian found that although it was easier to obtain quantitative results using scanning microanalysis, their new approach was able to detect extremely light elements and discriminate between isotopes; it also had greater resolving power.

In 1970, Pierre Galle used Castaing's and Slodzians' SIMS imaging approach to look at biological samples. He investigated renal tissue and red blood cells, observing that the distribution of sodium and potassium varied in different parts of the renal tissue. He also demonstrated that sufficient sodium was present in the red blood cells to produce a mass spectrometry–based 'picture' of the cell.

A further technical improvement, which would have a broad impact on imaging of biological samples using mass spectrometry, arrived in 1997. Richard Caprioli, Terry Farmer and Jocelyn Gile used matrix-assisted laser desorption/ionization (MALDI) for IMS for the first time to image peptides and proteins in tissues. The development of MALDI (**Milestone 18**) had transformed the analysis of large, biologically relevant molecules, but researchers typically combined and lysed cells prior to analysis, thus losing spatial information about where molecules of interest were originally located in the sample being analyzed. Caprioli and his colleagues took advantage of the high sensitivity of the MALDI technique (at that time, low-femtomole to attomole levels for proteins and peptides), recognizing that this would enable them to determine the molecular weights of molecules by analyzing small, discrete areas on a tissue surface. The researchers reasoned that coating a biological surface with a thin layer of the matrix required for MALDI-MS analysis and rastering the laser across the surface would enable point-by-point analysis of the molecules present at specific locations, much like the use of SIMS for elemental imaging. They found a diverse array of peptides and proteins in rat tissue samples, demonstrating the value of their method.

The interest in applying IMS in biology is reflected in the development of various commercial systems, mostly based on SIMS, desorption electrospray ionization (DESI; **Milestone 2**) or MALDI. Today, robotic methods are employed for speed and to obtain high-spatial-resolution images. Further biological applications are still under development, including the introduction of IMS into clinical pathology laboratories, suggesting that IMS will continue to be highly valued as an application of mass spectrometry.

Katharine Barnes, Managing Editor,
Nature Protocols

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