

Research news

Imaging with isotopes: high resolution and quantitation

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Mass spectrometry technology provides a clear image of the future of quantitative microscopy

"By the help of Microscopes, there is nothing so small as to escape our inquiry; hence there is a new visible World discovered to the understanding." Thus wrote Robert Hooke in his pioneering work *Micrographia* [1] published by the Royal Society in 1664. In this revolutionary book, Hooke described with excitement his discoveries using a simple light microscope, coining the word 'cell' to define the microscopic structures he saw in cork and plant samples. In this issue of *Journal of Biology* [2], Claude Lechene and colleagues describe a 21st century microscopy technology (Figure 1) that also reveals images we have never seen before.

Hooke was fascinated by the new vision of the world and the planets afforded by the lenses of the early light microscopes and telescopes of the 17th century. Ever since these discoveries, researchers have been gazing at the microscopic world and developing better and better instruments to do so. Over the centuries, the demanding needs of biologists have fuelled countless improvements in imaging technologies. For example, electron microscopy has become a standard instrument for high-resolution imaging (in the nanometer range) in biology, and scanning probe microscopy

techniques provide three-dimensional images of atomic surfaces.

Quantitative imaging with mass spectrometry

Lechene, of Harvard Medical School and Brigham and Women's hospital in

Boston, USA, knew exactly what requirements he was looking for in a quantitative imaging instrument. He was interested in using stable isotopes as tracers in biological samples. "To do that one has to be able to recognize them by **mass spectrometry**," explains

Background

- **Mass spectrometry** separates ions of different mass:charge ratios in order to analyze the composition of a sample.
- **Isobars** are nuclides (nuclei of atoms or atomic clusters) of the same apparent **atomic mass** (i.e. the same number of neutrons). **Isotopes** are different forms of the same element that have the same number of protons (the same atomic number) but different numbers of neutrons (different atomic masses).
- The **mass resolution** of a particular mass spectrometer is the smallest difference in atomic mass that can be distinguished by the instrument. The **spatial resolution** of a microscope is the smallest distance that can be resolved using it. The **sensitivity** of an instrument is the smallest amount of material that it can detect.
- **Sputtering** is the physical process whereby atoms in a solid target material are ejected into the gas phase as a result of bombardment of the material by a beam of ions. In **secondary-ion mass spectrometry (SIMS)** some of the sputtered atoms or clusters become ionized and can be analyzed according to their mass:charge ratios, to create a quantitative atomic mass image of the analyzed material.

The bottom line

- Multi-isotope imaging mass spectrometry (MIMS) has been developed from secondary-ion mass spectrometry (SIMS) by adding sophisticated ion optics, labeling with stable isotopes and quantitative image-analysis software.
- It is now possible with MIMS to monitor molecules labeled with stable or radioactive isotopes at a resolution and sensitivity that has not been possible with other techniques.
- MIMS can distinguish between ions of very similar mass, such as $^{12}\text{C}^{15}\text{N}^-$ and $^{13}\text{C}^{14}\text{N}^-$.
- Several isotopes can be imaged simultaneously using MIMS.
- MIMS can also generate quantitative images of atomic composition within subcellular compartments in tissue sections or cells without specific labeling.

Lechene (see the 'Background' box for explanations and definitions). "And there was no instrument to do so." During his studies in Paris, Lechene came across Georges Slodzian of the Université Paris-Sud in Orsay, a third-generation disciple of the French school of electron and ion optics. Slodzian's work on ion microscopy was a major input to the development of secondary-ion mass spectrometry (SIMS) [3], which is widely used in fields such as geochemistry, cosmology and materials sciences. "I needed an instrument that had high **spatial resolution**, the ability to detect several **isotopes** in parallel with high **sensitivity** and, at the same time, a **mass resolution** high enough to separate **isobars** like the ones found with nitrogen compounds," says Lechene.

The ability to look at multiple isotopes simultaneously was critical for assessing isotope ratios and normalizing one tracer isotope with respect to another; this is useful, for example, for distinguishing the isotope label from the endogenous atoms. The previous generation of instruments measured only one isotope at a time. Lechene's innovative

vision and Slodzian's technical wizardry led to the development of multi-isotope imaging mass spectrometry (MIMS) (see 'The bottom line' box for a summary of the technology). "Lechene was uniquely placed to make this development," notes John Vickerman of Manchester University, UK. "He is deeply immersed in the life-sciences community and has a long-standing interest in SIMS instrumental developments. Slodzian is an ion physicist of enormous skill and reputation who has been responsible for the ion-optical design of a number of extremely successful SIMS instruments. The new instrument that Slodzian developed has the spatial resolving power of an electron microscope with the added capability of detailed differentiation of chemical constituents."

Lechene's demanding requirements were important because he was keen to do experiments using the ^{15}N isotope. ^{15}N had been used for the pioneering experiments of Schoenheimer [4], to demonstrate protein turnover, and by Meselson and Stahl [5], to confirm the semiconservative nature of DNA replication. The problem is that nitrogen atoms hardly ionize and must therefore

be examined as cyanide (CN^-) ions. Lechene needed a system that could distinguish between the different isobars, such as $^{12}\text{C}^{15}\text{N}^-$ (mass 27) and $^{13}\text{C}^{14}\text{N}^-$ (also mass 27) and other similar atomic clusters. Slodzian's instruments enabled both high spatial resolution and the high mass resolution necessary for separating isobars at high secondary-ion transmissions.

Once the instrument and the tracer strategies were in place, the remaining challenge was developing the functional software and computational know-how to analyze all the data. Each image pixel has an intensity that is a function of the number of ions with a given mass that are at the pixel address. Lechene likens an image of 256 x 256 pixels to an array of over 65,000 test tubes. So, when the researchers analyze ^{12}C , ^{13}C , ^{14}N and ^{15}N , it's as if each of those test tubes contains four radioactive compounds. The isotope ratios are then normalized with respect to each other and then the peaks are analyzed. "When I began it took me weeks, if not months, to do some of the calculations. And now it takes us minutes," says Lechene (see the 'Behind the scenes' box for a summary on the development of MIMS).

A plethora of applications

Lechene teamed up with biologists from different disciplines to demonstrate how MIMS could be applied to quantitative imaging of biological samples. The Lechene study [2] is full of examples looking at turnover of proteins, DNA and fatty acids and at subcellular localization. Although these are spectacular examples of the MIMS technique, many researchers agree that this is just the tip of the iceberg. "The labelling of the lymph node cells by ^{15}N is really convincing and suggests that MIMS may be highly useful in immunology and cancer research," says Brad Amos of the MRC Laboratory of Molecular Biology in Cambridge, UK. "The paper shows that a remarkable amount of fine detail can

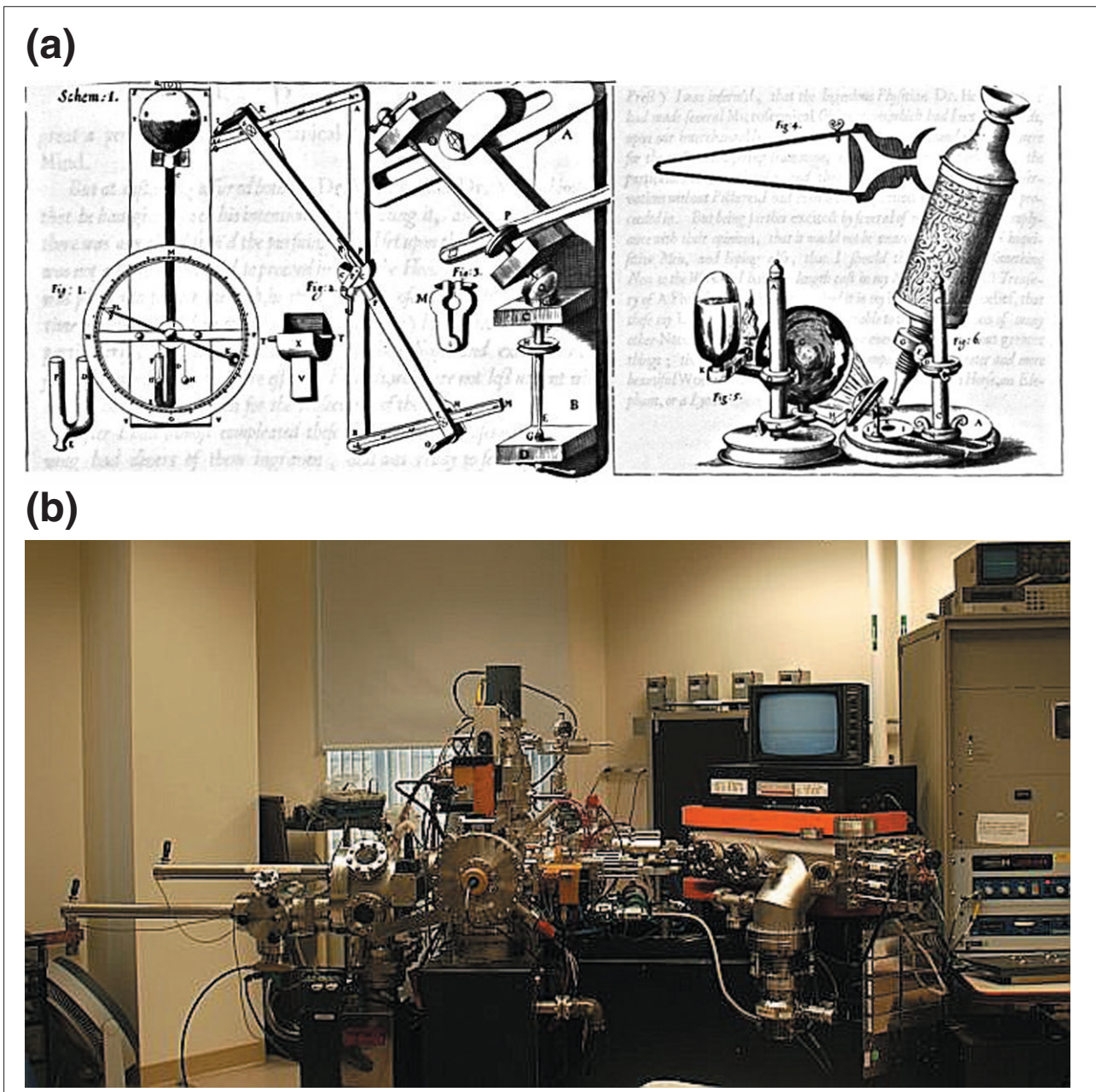


Figure 1
 Microscopy through the ages. (a) This illustration, from Robert Hooke's *Micrographia* [1], shows the plans for his lens-grinding machine and for his setup of the microscope. (b) Prototype of the NanoSIMS 50 (Cameca, France) used for MIMS technology.

be seen. This may turn out to be a key paper in the development of a really important imaging method."

"The most significant feature of this technique is that it opens up a whole new world of imaging; we haven't yet

imagined all that we can do with it," says Peter Gillespie from the Oregon Health & Science University in Portland, USA. He agrees with Amos that the technology represents an imaging revolution. "The novelty of the technique

means it will take some time for the details to be absorbed, [but it] sets a spectacular new standard for spatial resolution and detection of stable and radioactive compounds in cells." Vickerman is also enthusiastic about the

Behind the scenes

Journal of Biology asked Claude Lechene about the development of imaging mass spectrometry.

What prompted you to embark on the development of the MIMS technology?

It dates back to my MD studies in France and my work in biological research at the Commissariat à l'Energie Atomique (CEA) outside Paris. There I learnt and used tracer techniques with radioactive labels (radioactive sodium and potassium) coming from the nuclear reactor. I became interested in using isotopes as tracers and studied transport across cell membranes using electron probe microanalysis. There was a man there called Georges Slodzian who was developing ion microscopy technology. He finally invented the generation of instruments that offered the high spatial resolution I needed for biology and which was able to measure several tracers simultaneously. This allowed us to measure isotope ratios and do truly quantitative analysis on the system. For me, MIMS is not just an imaging instrument; for me, it is a measuring instrument - on this account it is unique. The imaging tells us where there is something at a subcellular resolution. But its real beauty is to be able to do precise quantitation. Suddenly, we have the ability to see and measure things that we could not see or measure before.

How long did the study take and what were the difficult steps you encountered?

Although we began dabbling with MIMS in 1998, these studies really took off in February 2003, after all initial difficulties with the prototype SIMS instruments were resolved and some quantitative image analysis software was developed. These advances resulted from a convergence of the work on secondary ion mass spectrometry in Slodzian's group and my experience in tracers and micromanipulation. We had to learn how to play with the samples, how to do the experiments with tracers and the calculations, etc. And the software development was the other essential part. The limiting parameter has become not the machine but the ability to analyze the reams of data that we get.

What was your initial reaction to the results, and how were they received by others?

When we finally got what I wanted, I found it even more exciting than I had ever imagined. And this has not stopped. For us it is marvellous; we see new stuff that no one has ever seen before and we really say "ooh!". People have begun to show an interest; it is a sigmoid increase and I think we are at the little shoulder of a very steep increase. You know what our colleagues are like when they don't know a method - and this one is difficult to understand. But there is more and more excitement in the biology community.

What are the next steps?

One avenue is metabolic studies and subcellular localization. This includes basic studies of transport of fatty acids to any position in the cell or monitoring the turnover of proteins, nucleotides or sugars in the whole cell in three dimensions. These applications go from basic science to diagnosis or drug localization studies. The second direction which we're pushing is the permanent or long-term labeling of cells to do cell-fate analysis. We have several ongoing collaborations to look at immunology, transplantation and stem cells. These are potentially quite exciting. We are also doing experiments where we take thousands of images of a single cell, slowly 'shaving' off layers from the top to the bottom, and then reconstruct them to create a three-dimensional representation of the entire cell, with distinct subcellular locations accessible for quantitative measurements.

applications: "This study is important in that it demonstrates across a range of demanding applications that SIMS can deliver unique information, inaccessible by other means."

But Vickerman adds a cautionary note about how widely MIMS technology will be applied in the future. "It is clear that the technique has great potential in medicine and biology, but there are two issues that have to be overcome: the conservative approach of much of the potential user community and the cost of the equipment." Gillespie agrees "Once commercial instruments are available, will they be affordable and easy enough to use that we will do many experiments with them? Or will they be like electron microscopes, where the expense of the instruments and the difficulty in operation means that relatively few people use them well?"

"It may take a long time (EM took a long time), but I am convinced that in 10-15 years this will be an easily accessible technique, with routine instruments in many departments of biological research," responds Lechene. A machine based on Slodzian's prototype is already sold by a French company called Cameca Inc., and Lechene notes that there are over a dozen around the world. At a cost of 2 million dollars they are beyond the budget of most laboratories. But Lechene is keen to point out that more and more mass spectrometry machines are being purchased. "After all, it's just the price of five electron microscopes, but it does so much more!"

Perhaps we should leave the last word to Hooke [1], whose prophecies echo through three centuries of improvements in microscopy: "Tis not unlikely, but that there may be yet invented several other helps for the eye, at much exceeding those already found, as those do the bare eye, such as by which we may perhaps be able to discover ... the figures of the compounding Particles of matter and the

particular Schematisms and Textures of Bodies.”

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