By Michelle Knott

On the Surface

Instruments provide scientists with the 'eyes and ears' they need to examine the world. Often such artificial 'senses' developed for one area of research can open up a whole new vista elsewhere. *Spotlight* examines how specialists in surface chemistry have developed a new instrument that is poised to change the way we look at biological samples.

Professor John Vickerman leads the team at UMIST responsible for developing the ToF-SIMS instrument shown here.

Professor John Vickerman and his team at the Surface Analysis Research Centre, UMIST, have spent decades developing instrumentation based on a technique called ToF-SIMS (Time of Flight Secondary Ion Mass Spectrometry). Today ToF-SIMS is a well established tool for looking at the surface of complex materials, both organic and inorganic, but it is also emerging as an effective way of imaging the biochemistry of cells. "The direct imaging of biochemicals within individual cells is an important target for biological research," explains Professor Vickerman. "Many intracellular processes are directional or localised to specific regions of the cell."

Techniques such as electron microscopy and fluorescence microscopy can investigate the distribution of proteins and other large structures inside a cell but smaller molecules play an important role in many biological functions and are more difficult to spot. "The development of ToF-SIMS to image the distribution of molecules in cells could provide a key experimental tool to probe the spatial organisation of intra- and inter-cellular processes," says Professor Vickerman.

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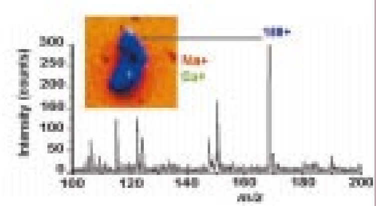
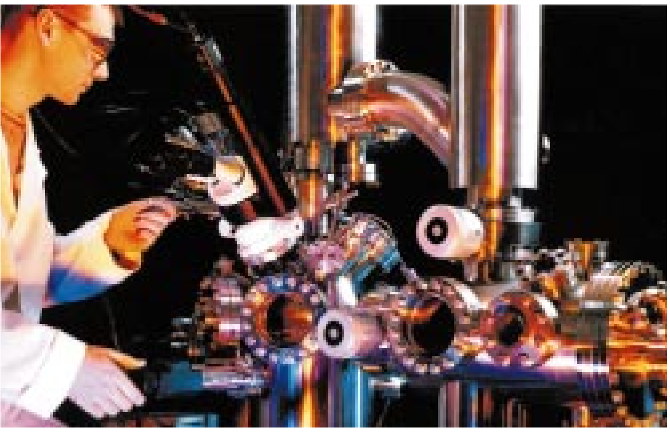


ToF-SIMS image of fractured prostate cancer cells showing overlay of sodium ions from the substrate (red), cellular phospholipid fragments ($C_4H_{11}NPO_4$, blue) and copper ions localised in the sub-cellular structure (green). Also shown is a partial mass spectrum from the fractured cells. The field of view is 100x100 microns.

Plume of atoms

ToF-SIMS fires a primary beam of ions at the surface of a sample to liberate a plume of secondary atoms and molecules – a process known as sputtering. Many of the sputtered species are neutral but some emerge charged as ions. These ions are then captured and analysed using mass spectrometry to build up a picture of the surface of the sample. The process sounds destructive but the trick to getting an accurate snapshot is keeping the dose of primary ions low, so that only one per cent of the surface atoms or molecules in the sample receive a direct hit during the experiment. This is known as 'static' SIMS because it essentially leaves the surface unchanged.

The basic SIMS process was invented in the late 1960s by Alfred Benninghoven at the University of Münster in Germany but it's an idea that has been adapted and improved in a number of ways in the intervening decades. In the early 1970s the SRC – the predecessor to SERC and EPSRC – began funding work on static SIMS at UMIST. The earliest instrument used a simple, quadrupole mass spectrometer to analyse the secondary ions. Subsequent versions were improved in the early 1980s, although they still relied on a quadrupole. The big breakthrough came at the start of the 1990s. When the SERC published its Achievements in Chemistry 1991, it included the UMIST group's work on ToF-SIMS as a way of improving the accuracy of the basic SIMS technique. By teaming static SIMS with time-of-flight mass spectrometry, Professor Vickerman and his colleagues had produced a technique that could effectively map the surface of a sample. This was the breakthrough that led to many international commercial laboratories installing SIMS systems as a way of analysing complex materials.



Left: Dr Nick Lockyer, researcher on the BioToF-SIMS project for the past six years.

Familiar names such as ICI, Bayer, Hoechst, Akzo, DuPont, Eastmann Kodak, IBM, 3M and Philips all adopted ToF-SIMS. It was also in the 1990s that the UMIST laboratory started to compile a library of static SIMS spectra so that today's analysts can compare unknown species with reference data.

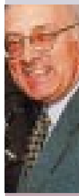
Naturally, UMIST wasn't the only place working on SIMS technology. An American group at Pennsylvania State University, led by Professor Nick Winograd, was also developing the technique and the two groups have a long history of co-operation. "We have shared developments very freely," says Professor Vickerman, "this has made the work very much more effective and ensured that we did not each reinvent the wheel." It was while Professor Vickerman was on sabbatical in Pennsylvania that the researchers together came up with the idea of applying the ToF-SIMS technology to biological samples: "Since ToF-SIMS operates by sputter desorption rather than thermal evaporation, chemicals with a very wide range of molecular weights can be analysed, making it possible to analyse biochemicals, biopolymers and pharmaceuticals," he told *Spotlight*.

The first stage was to develop a ToF-SIMS instrument with the required capability. A small UK company, Kore Technology, helped with the design and as a result of EPSRC funding in the UK and NIH funding in the US there are now cutting edge ToF-SIMS instruments working at both UMIST and Penn State. Each uses a liquid metal ion beam that can target an area smaller than 100 nanometres in diameter. However, handling biological samples presents a practical challenge – how to slice cleanly through a wet cell to expose a stable, unadulterated surface under the vacuum required for ToF-SIMS. The answer was to adapt the freeze-fracture process used to prepare clean samples for electron microscopy. "The freeze-fracture technique allows ToF-SIMS analysis of cells in their natural, hydrated state," says Professor Vickerman.

Room to improve

The UMIST team has already used the system successfully to scan liposomes – fatty acid spheres that provide a good model of cells – yeast cells (Candida glabrata) and prostate cancer cells. "The kit is close to being useable by biological scientists," says Professor Vickerman. There's still plenty of physical sciences research that can be carried out to improve the ToF-SIMS system however, and Professor Vickerman highlights three key areas: First, recent research suggests that using primary ion beams made up of large, polyatomic ions such as buckminsterfullerene can improve the yield of sputtered ions – especially larger molecules – from a surface. This could potentially make the system 1000 times more sensitive and UMIST is already building a buckyball-based system in collaboration with specialist firm Ionoptika. Secondly, the neutral particles in the sputtered plume may hold lots of information but they are currently lost. "Accessing this information using ionising laser pulses could provide new insights into the physics behind the technique and perhaps major sensitivity gains," says Dr Nick Lockyer, a member of the UMIST team. The third key target for improvement is in the analysis of the mass spectra. According to Professor Vickerman: "The spectral complexity which can arise when systems with multiple chemistries, such as cells, are analysed could result in an inability to see the wood for the trees. We are building spectral libraries but we also need computational systems that allow us to pick out the features that characterise chemistry differences between complex spectra."

Professor Vickerman believes that the key to the successful crossover of ToF-SIMS into the biological field lay in getting the right people together. For example, the researchers had enthusiastic input from their colleagues in biological sciences at both UMIST and Penn State. "In the US, Professor Andy Ewing had an interest in neurotransmitters and in UMIST, Dr Gill Stephens in bacteria. Both saw the potential and have produced stimulating problems," he adds, "it is the strong, collaborative, can-do approach of the group that has made it happen." □



Professor Nick Winograd of Pennsylvania State University has been working with the group at UMIST.

Contacts

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Much of the groundwork for using the instrument for cell analysis has been done by research student Ben Cliff.