chemistry in Australia
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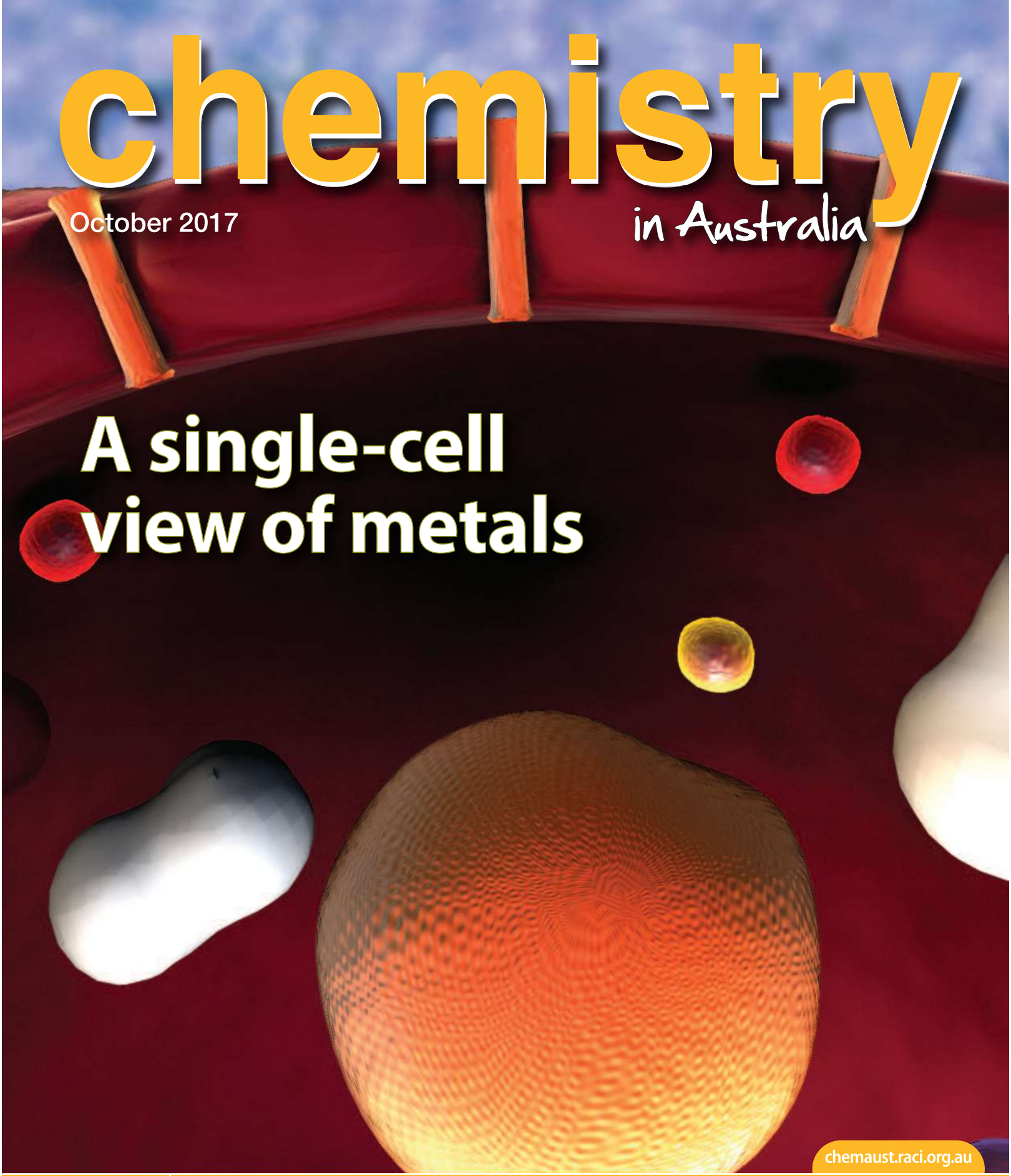
A single-cell view of metals



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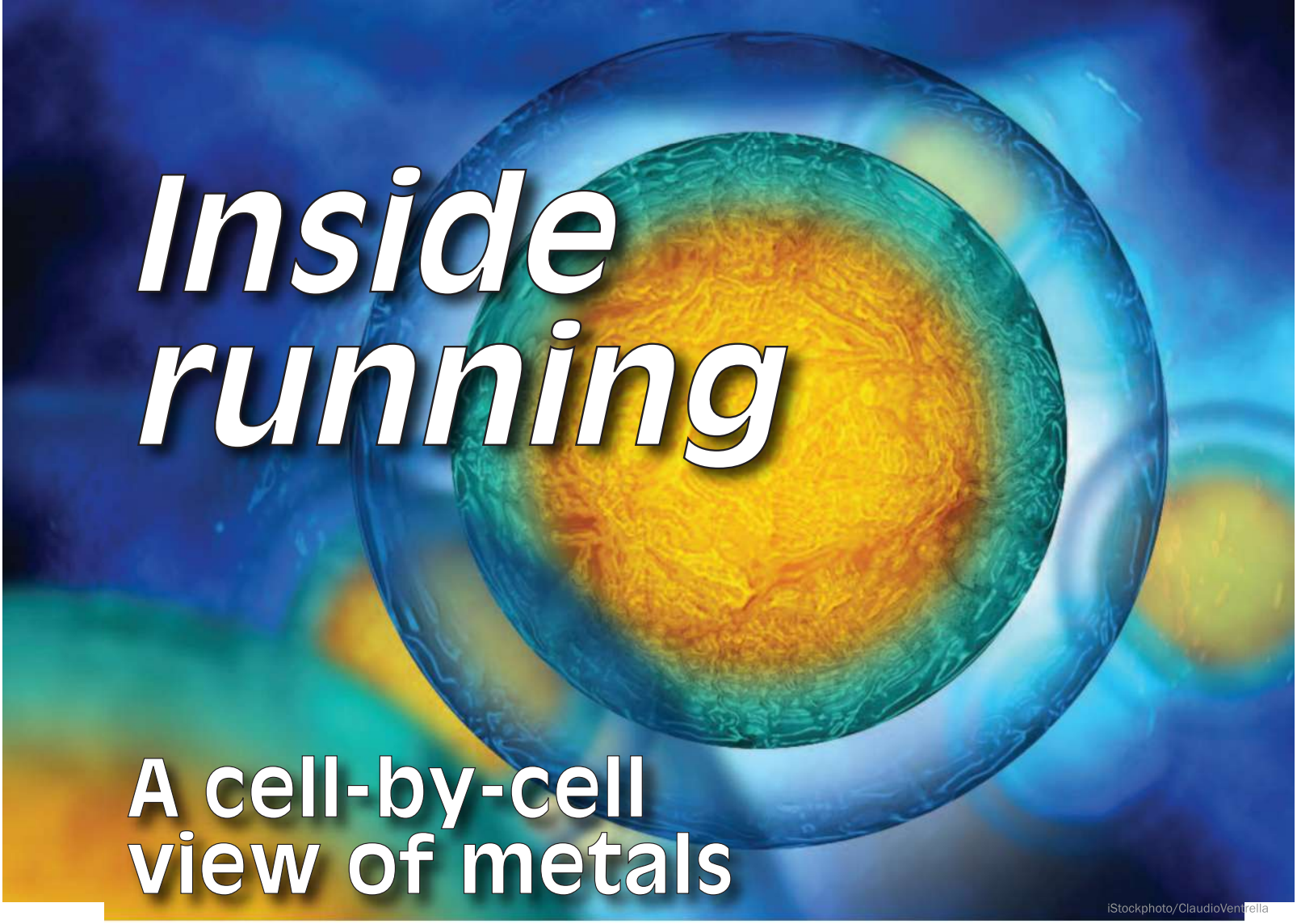

Inside running

A cell-by-cell view of metals

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Single-cell inductively coupled plasma mass spectroscopy offers a potentially significant breakthrough in detailed biological studies.

BY **DAVE SAMMUT**

'We're very interested in understanding metals in cells', says Dr Elizabeth New, Westpac Research Fellow at the University of Sydney. Metals in cells are potentially critical to cancer treatments, biological systems affected by environmental pollution, and the potentially deleterious effects of society's increasing exposure to nanoparticles.

ICP-MS (inductively coupled plasma mass spectroscopy) is well established as the go-to method for metals analysis. It has enabled researchers to identify correlations between metal levels and diseases, metabolic disorders, environmental exposures and nutrition.

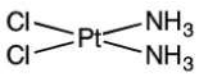
However, the technique has had its limitations, most particularly in the level of detail it can provide. Traditional ICP-MS methods digest the biological matrix, effectively capturing an average concentration across the sample. Until now, the technique has been unable to provide information about the concentration distribution across a biological cell population.

The Australian Institute for Nanoscale Science and Technology (AINST) at the University of Sydney is eagerly anticipating the delivery of the southern hemisphere's first single-cell ICP mass spectrometer from PerkinElmer. 'We already use ICP-MS very heavily, because it is very helpful in telling us about the uptake and accumulation of metals in cells,' says New, 'but this technology is really exciting because it will let us look at an individual cell and its metal content. That means that we can now look at different types of cells in a cell population and see exactly which has

When I heard about it, it was exactly what we needed for our research.

been specifically affected, by a disease or by a certain metal treatment.'

Cisplatin (*cis*-diamminedichloroplatinum(II)) is a commonly used chemotherapy drug used to treat multiple types of cancer. It is used to destroy rapidly growing cells by binding to the DNA and interrupting cell replication, so that the cell must repair the DNA damage or die. However, experience has shown that while many patients initially respond to this platinum-based treatment, the drug is less effective if there is a relapse and further therapy required.



cisplatin

One of three proposed mechanisms for this reduction in efficiency is that the cancerous cells evolve to have altered cellular accumulation (reduced uptake of the drug and/or increased export), but to date there has been little or no data about the distribution of the drug in the complex array of cells in a tumour. The existing ICP-MS techniques can only measure the average concentration across the whole cell population. Single-cell ICP-MS promises to measure the metal concentrations of individual cells down to the attogram (10^{-18} gram) level, to yield critical data about the metal distribution in the cell population, yielding up to 100 000 data points per second.

Using the first of the new instruments in the US, a team at the National Institutes of Health (NIH) has been looking at individual cell populations and their responses to platinum. By comparison, New and her team are eager to look at heterogeneous populations, with a view to how different cell types respond to platinum drugs. 'One of the real challenges in studying cancer is that the tumour contains so many different types of cells. If we can begin to identify certain cells in a tumour that have a particular metal fingerprint or a particular way of handling metals, then that will really help us think about how to treat tumours.'

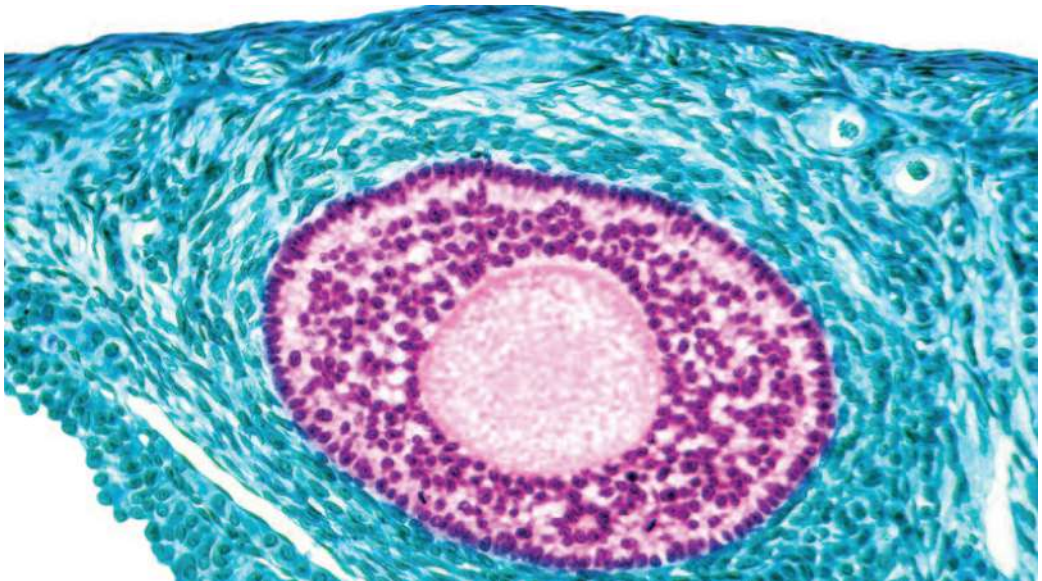
'We're interested in understanding the relationship between copper and platinum drugs – how cisplatin and other drugs affect copper in the cells', says New. 'And we're also interested in understanding oxidative stress in cells.'

'Oxidative stress is thought to be involved in pretty much every disease associated with ageing, but nobody knows whether it is the cause of disease or whether it is just an effect, whether we can measure oxidative stress and predict the outcome of the disease or response to treatment. If we can understand the interactions between the disease and the metal pools, or observe certain cell types with higher metal level and higher oxidative stress, then that would be a really exciting discovery.'

'It really was serendipity. I hadn't particularly looked for this, but now every time I think about something I think that this would be a great new use for the new equipment. It fits perfectly with what we're interested in. When I heard about it, it was exactly what we needed for our research.'

Sharing New's sense of anticipation is colleague Dr Wojtek Chrzanowski, senior lecturer and researcher at the Faculty of Pharmacy and the Australian Institute for Nanoscale Science and Technology. One key focus for Chrzanowski's team is nanotoxicity, based on the growing body of evidence that suggests that nanoparticles may have a causal role in various forms of disease, including cancer.

Nanoparticles occur in many products, including food, make-up, sunscreen, cosmetics, toiletries, baby formula and chewing gum. 'The main concern for us', says Chrzanowski, 'is the nanoparticles in food. One of the problems is that we are continuously exposed to these nanoparticles, but we don't know what exactly happens in the body and whether they have a toxic effect or not, and how they affect gut microbiota. There is already a lot of evidence that nanoparticles cause problems in our bodies, exacerbate



'The NexION SC-ICP mass spectrometer allows for the quantification of metal at the level of a single ovarian cancer cell' [pictured], Chady Stephan, Senior Leader of Applications at PerkinElmer and a co-researcher on the [NIH–PerkinElmer] ovarian cancer cell study, says. 'The technique is based on the ability to measure discrete signals generated from a cell when it enters the plasma, and allows for the quantification of cisplatin within individual cells', Dr Lauren Amable, NIH National Institute on Minority Health & Health Disparities, explains.

(Text and image: PerkinElmer)

symptoms of many diseases (e.g. colitis) and may even impact on the function of common drugs.'

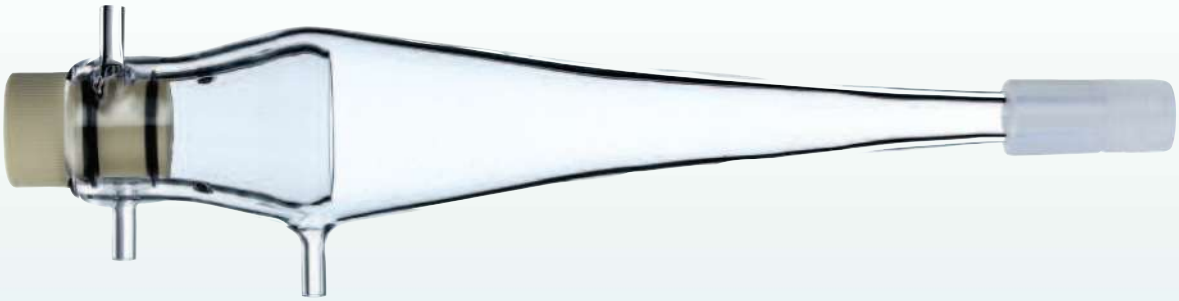
'There are a lot of factors – stress, contamination etc. – but we strongly believe that nanoparticles are one of the factors contributing to the increase in immune disease, asthma, autism, fertility problems or cancer, as well as an impact on the gut microbiota, thus the overall host community.'

Chrzanowski's expectation is that as nanoparticles are incorporated into cells, they consume critical energy as the cell seeks to consume or reject the particle, which interferes with the normal functioning of the cell. He then sees single-cell ICP-MS as a critical technique to allow his team to precisely screen for how many nanoparticles are taken up by individual cells, and how the cells change their normal metal concentrations in reaction to nanoparticle exposure. This aligns with and complements New's research questions.

Chrzanowski and his team are also looking at possibilities for the beneficial use of nanoparticles in targeted drug delivery. Malignant cells may take up nanoparticles and drugs like cisplatin differently, and the team would like to understand how this happens, why it happens, and how heterogeneous the responses really are. 'This instrument will allow us to put the nanoparticles onto the malignant and non-malignant cells and screen for how much of the nanoparticles were uptaken by which of the cell population. Based on this method, we should be able to design new drug delivery carriers.'

Another key functionality of interest

The single-cell ICP-MS instrument



One of the key challenges for single-cell ICP-MS is getting the cells into the plasma. Typical spray chambers (pictured) are designed to reject larger water droplets (>4 micrometres), but most biological cells are substantially larger than this.

As such, traditional ICP-MS techniques have involved digestion of the biological samples prior to analysis. PerkinElmer has developed its new Asperon™ spray chamber to incorporate new flow patterns that transport the larger single-celled organisms to the plasma, as well as limiting lysis (rupture) of the cells from impacts with the chamber walls.

However, prior to aspiration, the cells need to be washed and separated from the matrix, which may also contain the analyte. For example, a blood sample being tested for platinum distribution from cisplatin may contain the drug in both the serum and the cells. So the sample is first put through multiple wash and spin (gravity separation) cycles.

PerkinElmer's latest offering builds on the success of the NexION 350 ICP-MS instrument, introduced a couple of years ago. At just 10-microsecond dwell time, the company confirms that its high-speed detector gives it at least an order of magnitude speed advantage over its nearest rival. And this speed has been leveraged to create the capability to analyse biological samples at the single cell level of detail.

Let's take two groups of algal organisms as example. *Chroomonas* species have a typical size range of 5–7 micrometres. With a cell culture at about 250 000 cells/mL and a sample flow rate of about 15 µL/min, this gives a flow of approximately 63 cells/s, requiring a maximum read time of 16 microseconds for individual analysis, comfortably greater than the 10 microseconds claimed by PerkinElmer.

Gonyostomum semen is a much larger organism. A typical size of 50–70 micrometres, with a population of about 4500 cells/mL, translates to approximately 11 cells/s and a maximum read time of 89 microseconds. However, because of its larger cell size, this organism is more vulnerable to the higher pressures exerted at higher sample and gas flow rates, and PerkinElmer recommends a cell viability test prior to analysis.

According to Scott Fraser, National Product Specialist for PerkinElmer, 'I see a day coming where every medical institute (at least) will have one of these instruments, and this will become a routine part of personal, patient specific medical regimes – minimising the side effects of chemotherapeutic treatment and also maximising the efficacy of drugs within what we now understand to be heterogeneous populations of cancers.'

'There is also going to be a great development of understanding of the movement of metals through the biosphere', says Fraser, 'We'll better assess, per cell, how waste materials from various processes transfer in the food chain. That will become much more important as time goes on.'

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to Chrzanowski is the ability of the technique to distinguish the sizes of the nanoparticles. He expects that nanoparticles will aggregate, either within the cells or within the bloodstream. Being able to measure the size of particles in individual cells should then allow Chrzanowski to better understand the differing mechanisms and capabilities that relate to the cell's uptake of individual nanoparticles versus larger aggregates.

'The new single-cell ICP mass spectrometer will be a fantastic addition to our existing equipment, which can do both physical (size) characterisation and chemical characterisation. We have state-of-the-art facilities here at the University of Sydney that are still not available anywhere else in Australia. We will link it together to create a nano toolbox which can probe nanotoxicity and response from different angles to give the full picture.'

Dave Sammut FRACI CChem is principal of DCS Technical, a boutique scientific consultancy, providing services to the Australian and international minerals, waste recycling and general scientific industries.

Follow that cell: an NIH challenge

In June, the US National Institutes of Health named two biological engineering researchers as winners in phase 2 of its Follow that Cell Challenge. The winners will share $400 000 in prizes awarded for development of new tools and methods for predicting the behaviour and function of a single cell in complex tissue over time – and how that reflects the health of the tissue. They were chosen from among several phase 1 finalists.

Dr Nader Pourmand, University of California Santa Cruz, is the first place winner with a prize of $300 000. A team led by Dr Paul Blainey, of the Broad Institute, Cambridge, Massachusetts, will share the second place prize of $100 000.

Pourmand developed an advanced 'nanopipette' technology with such a fine tip that it makes it possible to non-invasively sample tiny amounts of intracellular material to measure biochemical changes, multiple times in the same cell – without disturbing its function. Coupled with parallel development of 'nanogenomics' technology, this will enable scientists to track molecular changes in cells that develop in response to treatments, such as the development of drug resistance in cancer.

'This is the only technology I know of that enables us to repeatedly interrogate a single cell without killing it', said Pourmand.

Blainey's team designed a new molecular technology to streamline cellular analysis and allow for wide adoption by labs. Instead of requiring complex physical sampling of cellular components, it takes advantage of cell secretion pathways to access molecules of interest inside the cell – at multiple time points. Blainey's findings demonstrated the ability of a cell to 'self-report' gene expression.

Launched in August of 2014, Follow that Cell seeks to incentivise innovation by awarding prizes on the basis of completed work, in contrast to NIH's traditional, prospective grant and contract mechanisms, which provide funding in advance.

The challenge is aimed at finding new ways to learn how cells transition from a healthy to a diseased state, become responsive to treatment, and offer opportunities for early detection and precision medicine.

In March 2015, NIH announced selection of 16 finalists from phase 1 of the competition, which sought proposed theoretical solutions rather than hands-on experimentation. All of the phase 1 finalists were eligible to participate in phase 2, during which they were encouraged to execute their ideas in proof-of-concept studies.

'The prize winners have brought some creative ideas to the study of single cells', said NIBIB director Roderic Pettigrew, 'We challenged competitors to develop new ways to monitor biochemical changes in individual living cells and they have demonstrated an impressive capacity for problem solving and innovation.'

Read the winners' own brief descriptions of their prize-winning solutions at bit.ly/2sSuvOf.

National Institutes of Health