Customer Insights

- How Mass Spectrometry Techniques are Propelling the Advancements of Single Cell Biology

Mass Spectrometry

Innovation with Integrity

How Mass Spectrometry Techniques are Propelling the Advancements of Single Cell Biology

Bruker's range of high-spec mass spectrometry instruments enable the Sweedler group at the University of Illinois at Urbana Champaign (UIUC) to take the lead in analytical discovery

Working with Bruker
Prof. Jonathan Sweedler's research group at UIUC benefits from a broad range of analytical equipment chosen for its optimal performance for his specific applications.

"For mass spectrometry imaging, Bruker's technology leads the way. In particular, their MALDI imaging instruments have incredible figures of merit that other vendors don't come close to."

The Department of Chemistry at UIUC

The Department of Chemistry has been a part of the University of Illinois at Urbana Champaign (UIUC) since its establishment in 1868 and supports a range of research areas from chemical biology to analytical, inorganic, materials, organic, physical and theoretical chemistry. Consistently placed in the top of national chemistry department rankings, UIUC is in a prime position for innovative chemical discovery.

Professor Jonathan V. Sweedler, a faculty member of the department for almost 26 years, conducts bioanalytical chemistry research at the intersection of analytical chemistry, bioengineering, neuroscience, and physiology. With over 365 peer-reviewed publications to his name, Professor Sweedler is a leader in the field; his group currently consists of two research scientists, 7 postdoctoral associates, 12 graduate students, two visiting scholars and 11 undergraduate students. With a research focus on single cell biology, his group relies on robust instrumentation to make ever-increasingly detailed measurements of small biological samples, such as individual cells, related to a broad range of fundamental and applied studies.

The Research

The work done by the Sweedler research group can be broadly categorized as measurement science and technology, with a focus on the development of analytical methods for investigation of complex biological specimens. The Sweedler lab is renowned for developing, optimizing, and using highly sensitive methodologies to study a wide range of model organisms, spanning many phyla, with an overarching goal of gaining a better understanding of known neurochemical pathways and discovering new and unusual pathways. Sweedler is especially interested in discovering and annotating novel neuropeptides involved in mechanisms of behavior, memory and learning. By using microscopy-guided MALDI (matrix-assisted laser desorption/ionization) mass spectrometry

and capillary electrophoresis-coupled electrospray mass spectrometry, cells can be probed for cell-to-cell signaling molecules to map the chemical bases of connectivity within well-defined neuronal networks.

The Sweedler group has chemically characterized thousands of neuropeptides across a wide range of animal models, through a combination of analytical approaches.

"It is a rather bold statement, but we have discovered and reported more novel brain peptides across the animal kingdom than any other group. This extends to discovering novel neuropeptide genes as well," comments Professor Sweedler.

An increasingly important area of research is that of single cell measurements. By developing and using sensitive and low sample-volume methods, the genes expressed by an individual cell type can be elucidated, and the dynamic phenotypes of seemingly homogenous cells under different external situations can be characterized and classified. Professor Sweedler describes the nature of his groups' neurobiology research with mass spectrometry imaging:

"We want to know what compounds are in the brain, where they are and when they were at particular location, as well as how they change based on behavior, learning and environment. Mass spectrometry shows you what compounds are present in the brain, for example, by removing a brain region and assaying it with liquid chromatography mass spectrometry. We also want to know where the compounds are, and so we can take a tissue slice from the brain, conduct mass spectrometry imaging, and figure out the localization of different chemicals. For example, we can ask what happens in distinct brain regions of rats when you alter their diet, or induce learning with a learning task. We perform the mass spectrometry imaging measurements with a Bruker ultrafleXtreme™ and our new solariX XR™. These instruments allow us to probe what's in a particular location, for example within a layer of the hippocampus, and characterize the changes in neurotransmitters, neuropeptides, and lipids"

What the Group works on

"Single cell measurements are an exciting area. In the last few years, there has been an increase in interest and government funding channeled towards this technology, and therefore it is a hot area that scientists – undergraduates, graduate students and post docs – want to work in. ...

... Because of this interest, around half of my group is now working in single cell biology, using some of the newest equipment we have, such as the solariX XR™."

Sweedler's group is constantly striving to improve the sensitivity and information content of their measurements, which goes hand-in-hand with access to the best instrumentation. The solariX XR™ provides this level of information through extremely high mass resolution detection.

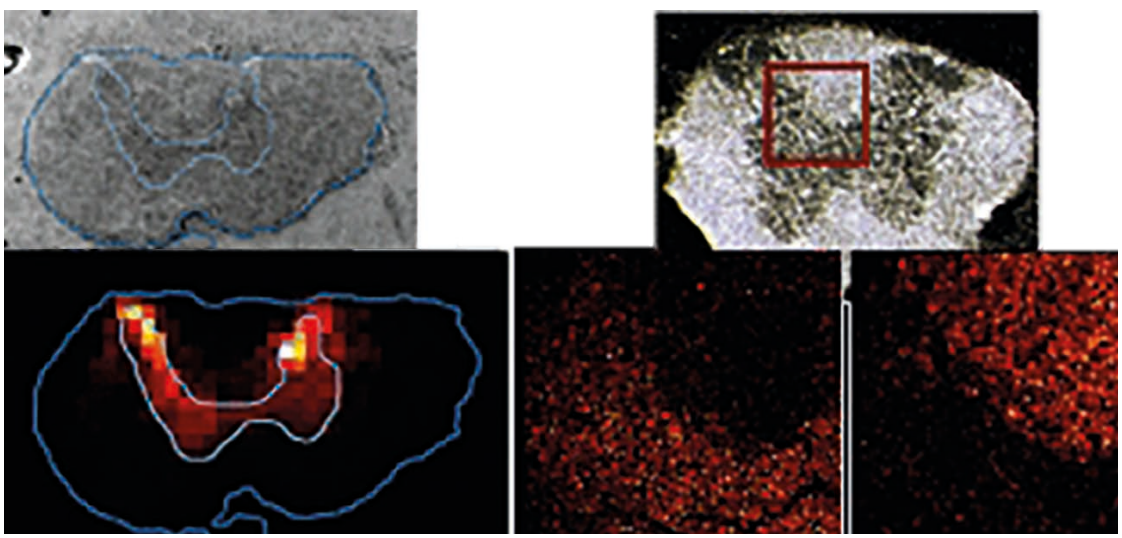
One challenge in academics that is not found in industry is the need for continuous training. In any academic setting, the rate of staff turnover is faster than in industry as the undergraduate, graduate and postdoctoral associates move on to other positions every few years. Although this is a constraint on the level of expertise that can build up within the Sweedler group over time, this certainly helps the field in general. For example, perhaps 50 percent of Professor Sweedler's students move into the pharmaceutical industry, bringing their expertise to many other teams. A benefit to a steady influx of students and postdoctoral associates is that *"new people tend to be fairly fearless and want to try new things,"* according to Professor Sweedler.

While there is a steady turnover, he has been fortunate to have two senior scientists, Dr. Stanislav Rubakhin and Dr. Elena Romanova, who have been in the group for about 20 years.

"One of our goals is to carry out complex measurements on cell-to-cell signaling, for example, on neurotransmitters and neuromodulators across the brain. It has been enabling to have these two expert senior scientists with broad expertise in sample preparation, LC mass spectrometry, mass spectrometry imaging, and a myriad of other related areas work on these projects. They contribute to the long-term group knowledge of instrumentation and procedures and enable a number of projects to succeed."

Training

The level of training provided to students and staff in the group is vital for excellence in research done inside of the group and in multiple collaborative projects, as well as future contributions from



group members who move into science and industry. Members enter the lab begin with varying degrees of knowledge and experience with mass spectrometry, so the training requirements vary. Professor Sweedler explains,

"Single cell measurements with mass spectrometry require a steady hand, and potentially someone who doesn't drink much coffee! We have found it best to train undergraduate students on equipment like the MALDI mass spectrometer where small samples can be used and the instrument is robust. For example, the ultrafleXtreme™ can handle a large number of sample plates, and so is great for training. If students want to progress to capillary electrophoresis - mass spectrometry, this makes a good transition for more experienced users as more attention to detail and greater potential for complications exist."

As well as receiving training from higher level staff and academics, Bruker has provided multiple training events and continuous support for the Sweedler group, enabling them to sustain a high degree of knowledge on the latest equipment required for cutting-edge research.

"Continuous training is one of the beneficial elements of collaborating with Bruker," ...

... says Professor Sweedler, adding that "*they supply an incredible amount of expertise – that's worth a lot!*"

Bruker also benefits from this relationship; of the scientists that come from Sweedler lab, around 50 percent head towards the pharmaceutical industry, bringing with them their affinity for Bruker instruments.

A Challenge for the Group

When conducting research at the individual cell level, there are certainly many challenges to overcome. Professor Sweedler identified several hurdles to cross before single cell measurements become robust: more universal sampling protocols, increasing analyte detectability, and improving the throughput of mass spectrometry.

"My group is continually fighting against detectability, attempting to achieve progressively lower detection limits. We have pushed creating better sampling approaches and we are using instruments where we can work at the single cell level and have the best detection limits – ...

... in practice this has always tended to be with Bruker, because of their increasingly sophisticated mass spectrometry technologies."

There are a number of unusual physical constraints that they face. Professor Sweedler explains how his team overcame one such challenge:

"Studying brain chemistry involves sacrificing animals and removing brain tissue. If someone has a genetically modified mouse that they want us to take measurements on, it is difficult to send cells that stay intact overseas. We have a room on our campus that can be sterilized, where we can complete the dissection and run samples without having to quarantine the animals. In another recent example, we were collaborating with someone in the UK who was researching salt loading in animals and how this affects a certain brain region. His work was mostly conducted on rodents, but he also had a partnership with scientists in the Middle East completing similar studies on camels. The first set of samples had too high a measurement variability and so one of my senior scientists went to the Middle East to help them collect more rigorous and quantifiable samples. We find we often have to work with those collecting the samples and train them to obtain the best quality data."

The Takeover of the Sweedler Group by Mass Spectrometry

At the start of Professor Sweedler's career at UIUC, there were a number of analytical techniques used to conduct measurements on samples, of which mass spectrometry was one. However, mass spectrometry methods and equipment have sustained significant improvement and growth, and this is taking over other areas. Mass spectrometry is becoming an even more potent tool in the hands of scientists when combined with other techniques such as transcript measurements, electrophysiology and fluorescent microscopy. Professor Sweedler described how analytical measurements have changed over the 25+ years since he began at the university:

"My initial research proposal was on how to measure cell-to-cell signaling, in the brain. I proposed working with sea slugs, a simple organism with 10,000 neurons that can learn to find food and avoid being eaten, which we still work on today. We also now work on both simpler and more complex animal models, the more complex being rodents and sometimes primates. Twenty-five years on, we're more capable of getting information out of our samples and conducting measurements, and equipment has entirely changed. Back then I proposed to use capillary electrophoresis with fluorescent detection, in addition to radioactivity detection and NMR. Now almost all measurements involve mass spectrometry. ...*

... Somewhere along the way we started working with Bruker and as mass spectrometry improved, other methods have been used much less often."

New Instruments for new Measurements

The world of single cell biology is rapidly gaining traction and will continue to do so in the coming years. This alone will help facilitate advancements in the technology required to make these measurements. As one recent development, the Sweedler group has devised a high throughput approach where they disperse thousands of cells on a microscope slide, make them fluorescent, and use optical microscopy to identify cell locations. The information on the specific locations guides the Bruker ultrafleXtreme™ and the solariX XR™ to acquire mass spectra from only the cells and not the empty spaces between them. This enables them to obtain lipid and peptide profiles from tens of thousands of cells.

As another area of research, the Sweedler group is working to combine mass spectrometry with other chemically information-rich approaches:

"We're working with a couple of groups where we can image a brain slice with vibrational spectroscopy such as infrared and Raman. Light is non-destructive, so once we get that chemical information, we can then carry out mass spectrometry imaging. We are currently working on how to combine the different datasets."

In addition to this, the group is also developing an approach for sampling brain slice content after mass spectrometry imaging at select locations, and then performing capillary electrophoresis mass spectrometry analyses of collected analytes.

**For more details visit:*
www.nsf.gov/news/special_reports/science_nation/sluggishthoughts.jsp

How has Bruker's Technology helped to solve these Challenges?

When assessing which instrumentation should be used for a particular experiment, there is not always a clear-cut answer.

"Sometimes one instrument will excel, and another will not," explains Professor Sweedler. *"There are several flavors of instruments, but when it comes to mass spectrometry imaging with MALDI MS, Bruker has very strong platform performance."*

Bruker also provides an abundance of expertise, from software update assistance to on-site training at UIUC, and *"offers a partnership which is worth a lot!"* – adds Professor Sweedler. Bruker also facilitates the way in which Professor Sweedler pushes their instruments to higher and higher limits:

"*We often use the instruments slightly differently than most users, which can be tricky, but Bruker has always helped us.*"

"*One job of an academic analytical chemist is to push the measurement science beyond what it can easily do. A frequent question we ask ourselves is 'we want to make this measurement, but how can we make the instrument do it?' – working with Bruker enables us to collect such data.*"

Professor Sweedler's lab recently acquired Bruker's solariX XR™:

"If I had to pick one instrument to save in a fire (and did not have to worry about its size or weight), it would be the solariX XR™. ...

... It's a lower throughput instrument than the ultrafleXtreme™, but it provides great chemical information with the added capability of electrospray."

Future

Solutions to the challenge of single cell measurements have shifted over the past two decades. Professor Sweedler explains that:

"Companies like Bruker are enabling for single cell measurements because they are producing increasingly high quality mass spectrometers. ...

... It's kind of a fun evolution! The last two years have been especially exciting with the combination of mass spectrometry with other approaches."

"In the next decade, I would like to see more robust methods of data integration with other non-mass spectrometry based platforms. We can currently acquire a large amount of data, but the ability to integrate data not just with Bruker instruments but with other instrumental platforms as well, really needs to be improved. Data integration tools need to become more universal."

The longevity of the Sweedlers group collaboration with Bruker is a testimonial to Bruker's high specification equipment, which allows labs like Professor Sweedler's continue to make revolutionary discoveries, through pushing the instrumentation to its limits.

References:
1. National Science Foundation, 2016. Sea slug brain chemistry reveals a lot about human memory, learning
www.nsf.gov/news/special_reports/science_nation/sluggishthoughts.jsp
[Accessed 16/01/2017]

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