



*The*  
chemical  
cartographer

*Pieter Dorrestein  
is using mass  
spectrometry to map  
out the microbial world.*

BY PAUL TULLIS

A part from the treadmill desk, Pieter Dorrestein's office at the University of California, San Diego (UCSD), is unremarkable: there is a circular table with chairs around it, bookshelves lined with journals, papers and books, and a couple of plaques honouring him and his work.

But Dorrestein likes to offer visitors a closer look. On his computer screen, he pulls up a 3D rendering of the space. Four figures seated around the table — one of whom is Dorrestein — look as if they've been splashed with brightly coloured paint. To produce the image, researchers swabbed every surface in the room, including the people, several hundred times, then analysed the swabs with mass spectrometry to identify the chemicals present.

The picture reveals a lot about the space, and the people in it. Two of Dorrestein's co-workers are heavy coffee drinkers: caffeine is splotched across their hands and faces (as well as on a sizeable spot on the floor — a remnant of an old spill). Dorrestein does not drink coffee, but has left traces of himself everywhere, from personal-care products to a common sweetener that he wasn't even aware he'd consumed. He was also surprised to find the insect repellent DEET on many of the surfaces that he had touched; he hadn't used the chemical in at least six months.

Then there were signatures of the office's other inhabitants: the microbes that reside on human skin. Dorrestein has been using mass spectrometry to look at the small molecules, or metabolites, produced by these microbes, and to get a clearer picture of how microorganisms form communities and interact — with other microbes, with their human hosts and with the environments that they all inhabit.

He has analysed microbial communities from plants, seawater, remote tribes, diseased human lungs and more, in an effort to listen in on their chemical conversations: how they tell one another of good or bad places to colonize, or fight over territory. The work could identify previously unknown microbes and useful molecules that they make, such as antibiotics.

"The applications are broad," says Katie Pollard, a comparative genomicist at the Gladstone Institutes at the University of California, San Francisco. Because many microbes cannot be cultured and studied directly, she explains, "these approaches that assay them *in situ* are totally game-changing". They also directly address some of the main goals outlined in the US\$521-million National Microbiome Initiative, announced by the White House's Office of Science and Technology Policy last month. Dorrestein was present for the announcement.

In this fast-moving field, Dorrestein has set himself apart by building useful tools and productive collaborations. "Pieter is genuinely interested and very creative," says Janet Jansson, division director of biological sciences at Pacific Northwest National Laboratory in Richland, Washington. In April, she visited UCSD, and Dorrestein asked whether he could swab her hand for one of his studies. "I said, 'Oh! I want to do that! I want to be involved in that study!'" Jansson recalls: "It's interesting and exciting science that people want to participate in."

## ROCK AND ROLL

Dorrestein grew up in the Netherlands, and became obsessed with rock-climbing when he visited family friends in Tucson, Arizona, at the age of 16. Faced with the flatness of his homeland, he applied to Northern Arizona University in Flagstaff, in large part because of its proximity to the many stone towers of the Four Corners region, where Arizona meets New Mexico, Colorado and Utah. He studied geology and chemistry, but intended to pursue his passion for climbing. Shortly after graduating in 1998, however, an experience on the 900-metre-tall face of El Capitan in Yosemite, California, made him think again.

He was clinging to the rock about 50 metres above his last anchoring point, and realized that if he were to lose his grip, he would drop

100 metres before his safety line tautened and slammed him into the granite. It wasn't fear, he says, but rather his lack of it that troubled him. "I thought, if I keep doing this, it won't be a good ending," he recalls. "So I rappelled down."

Pieter Dorrestein's methods could reveal what microbes do in complex communities.

He drove home to Flagstaff that day, and started filling out applications to graduate school. He ended up at Cornell University in Ithaca, New York, studying how microbes produce small molecules such as vitamin B1. It was here that he was first introduced to mass spectrometry.

Mass spectrometry generally involves breaking complex molecules apart, ionizing them and measuring the mass of the resulting fragments, which can be used to calculate the composition of the starting molecules. Dorrestein uses the analogy of a bar code — mass spectrometry creates a unique identifier for each chemical in a sample.

Spurred by his interest in the technology, he went on to do a post-doc in the lab of Neil Kelleher, a chemical biologist at the University of Illinois at Urbana-Champaign. Kelleher was pioneering efforts to do 'top-down' mass spectrometry, in which intact, rather than digested, proteins are put directly into the mass spec. The approach allows researchers to identify small modifications made to proteins, but the process is slow. Within two months of his arrival in Illinois, Dorrestein had developed a speedier approach that allowed him to examine certain large enzymes systematically<sup>1</sup>. "We boiled down years of work into days, basically," Dorrestein says. He ended up co-authoring 17 papers in 2 years. "Pieter has that unusual combination of creativity and drive, along with an incredible ability to finish projects," says Kelleher, who is now at Northwestern University in Evanston, Illinois.

Dorrestein joined the faculty at UCSD in 2006 — but things really kicked off for him when Palmer Taylor, then dean of the university's school of pharmacology, authorized the purchase of a MALDI-TOF mass spectrometer (matrix-assisted laser desorption/ionization time of flight), which would allow Dorrestein to do mass-spectrometry imaging. "That changed the whole world around," he says.

## SPACE CRUSADERS

As well as identifying molecules in a sample, mass-spectrometry imaging provides spatial information. MALDI-TOF uses a laser to heat up and ionize molecules. By scanning that laser across a 2D sample, researchers can capture an 'image' that shows exactly where different molecules in the sample reside. The technique can be used to identify and locate biomarkers in slices of tumours, but with his interest in microbes, Dorrestein wondered whether he could take colonies of bacteria on a Petri dish and scan them directly to see the metabolites they produce.

No one had ever tried it. Dorrestein suspects that they were afraid of getting their expensive mass spectrometers dirty — "and this is as dirty as it comes, putting microbes directly into the instrument". So he tried a simple experiment, asking an undergraduate student, Sara Weitz, to scan a colony of *Bacillus* bacteria.

The images generated "weren't the prettiest", Dorrestein says, but they indicated that the process worked. He sent them to Paul Straight, a microbiologist who had just joined the faculty at Texas A&M University in College Station. "I'm pretty sure his jaw dropped," Dorrestein says. Together, the two teams used mass-spectrometry imaging on colonies of *Bacillus subtilis* and *Streptomyces coelicolor* grown next to one another. By exploring the spaces where the colonies interacted, they were able to identify molecules that the microbes use to compete with each other<sup>2</sup>.

Actually visualizing this microbial arms race, Dorrestein says, makes him think back to 1928, when Alexander Fleming isolated penicillin from a mould that was killing bacteria on a dish. Mass-spectrometry imaging could quickly reveal the chemistries of such interactions, and perhaps speed up the search for new antibiotics.

Dorrestein decided to shift his lab to focus almost exclusively on these methods. He was still an early-career investigator, and almost everybody he knew discouraged him from taking such a big risk. But Taylor pushed him to apply for tenure right away. "Pieter's potential to think outside the box in the analytical and computational arenas was immediately evident," Taylor says. "His research took off very rapidly."

The problem with looking at dirty samples is that they produce messy data. Scanning microbial landscapes produces thousands of bar

codes, but it's largely unknown what they correspond to; they haven't been annotated. "It's the equivalent of looking under the lamp post," Dorrestein says: one can only 'see' the molecules that have been identified before, and the vast majority haven't. This is currently a big challenge for the field, says Jansson. "It's possible to analyse features by mass spec, but still very difficult to identify what those features are."

To help to make sense of the heaps of data, Dorrestein worked with Nuno Bandeira, a computational biologist at UCSD, on an approach that classifies bar codes and the molecules to which they correspond according to their relationships with other annotated molecules<sup>3</sup>. This allows researchers to start predicting, computationally, the structures and functions of thousands of metabolites. But there's still a dearth of annotation: although thousands of people worldwide conduct mass-spectrometry research, most annotate only the few molecules that they're interested in.

So, beginning in 2014, Dorrestein and graduate student Mingxun Wang from Bandeira's lab started to develop a way to crowdsource annotation. They launched the Global Natural Product Social Molecular Networking website, a repository and data-analysis tool that enables researchers to uncover relationships between related molecules, group similar ones together and compare data sets. "This is something he's brought to the field that has really helped," says Jansson.

### TEAM WORK

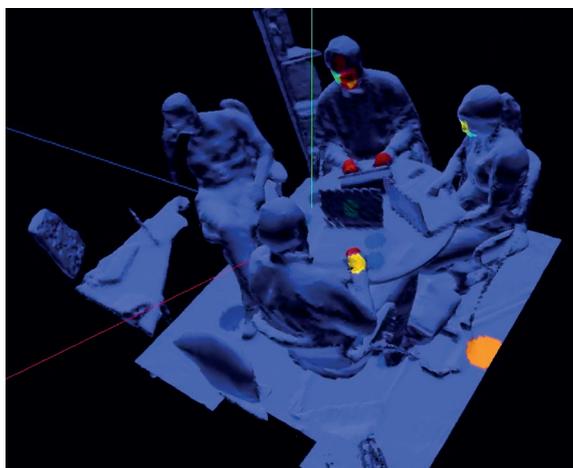
One of the keys to Dorrestein's success has been his collaborations. Rob Knight, a leader in microbiome DNA and RNA sequencing, works just across the quad from Dorrestein's office. They've teamed up to blend sequencing with mass spectrometry. Last year, a postdoc in Dorrestein's lab, Amina Bouslimani, took swabs from one male and one female volunteer, at 400 spots on their bodies — twice. One swab from each spot went to Knight's lab so that the microbes in it could be sequenced, and the other went for mass spectrometry to identify the chemicals, natural and artificial, that coexist with the microorganisms.

The participants had refrained from showering or using cosmetics for three days, but the chemical signatures from the hundreds of different types of microbe in the samples were overwhelmed by chemicals from beauty and hygiene products<sup>4</sup>. Still, the researchers did find correlations between microbe communities and local chemistries: for example, the bacteria found in the vaginal area were correlated with molecules associated with inflammation. Such connections, Dorrestein says, could be used to generate hypotheses about host–microbe interactions.

Bouslimani is now analysing samples from volunteers' hands and from personal items such as their mobile phones. The work, which has not yet been published, has shown that people leave persistent chemical signatures on the objects that they touch — like those in the image of Dorrestein's office.

Bouslimani and Dorrestein think that this could have applications in forensic science. A suspect could be swabbed to determine whether the chemical signature of his or her skin matches that at a crime scene. Or in the absence of DNA or fingerprint evidence, the chemicals that a criminal leaves behind could help to provide a lifestyle profile: a composite sketch of the products that they use and the mixture of microbes they carry. "Maybe the chemical signature can help the investigator narrow down who was there," says Bouslimani.

Last year, Dorrestein teamed up with microbiologist Maria Dominguez-Bello of New York University and several others who wanted to see what human skin and its microbial diversity look like when people grow up free of the trappings of the developed world. They



Caffeine appears as coloured spots in a 3D visualization.

collected samples from some remote tribes — one near Manaus, Brazil, and Tanzania's Hadza people — and compared them with swabs from non-tribal people near the collection sites. Using Dorrestein's mass-spectrometry techniques, they've found that people in the tribes have more-diverse microbial communities and skin chemistry than those living a more modern lifestyle. The ongoing work is serving up some surprises too, says Dorrestein. People from one village in Brazil had a range of pharmaceuticals on their skin, indicating that they had more contact with outsiders than previously suspected.

Dorrestein has a way of leaning forward and almost standing on his toes in excitement when he talks about

the technology and how it might help to assess the health of oceans, or improve efficiency in agriculture, a major contributor to greenhouse-gas emissions. But when asked how he chooses projects to pursue, it's work on human health that he mentions first. "To us, that's a really obvious, direct application of this — we want to help patients," he says.

Dorrestein teamed up with Knight, Doug Conrad — director of UCSD's adult cystic fibrosis clinic — and others to develop a rapid microbial diagnostic test. Cystic fibrosis causes a build-up of mucus in the lungs, which can periodically become infected with bacteria. These infections require aggressive treatment with antibiotics — and sometimes the bacteria can develop resistance. Dorrestein and his collaborators have shown<sup>5</sup> how analysing mass-spectrometry data on a phlegm sample from someone with cystic fibrosis can identify microbial communities that standard medical culturing techniques miss.

Louis-Félix Nothias-Scaglia, a postdoc who joined Dorrestein's lab this year, is mapping the skin of people with psoriasis, a condition thought to be triggered by an overactive immune system. If molecules produced by certain bacteria are present when the condition flares up but not when the skin is healthy, Nothias-Scaglia explains, they might point to drugs that could treat or even prevent the disease. Even being able to use microbial changes to predict when a flare-up is coming would enable patients to reduce their use of immune-suppressing drugs.

Turning such data-intensive techniques into standard lab tests will be a challenge. "Cynics would say it's too complicated, it's never gonna go anywhere," says Conrad. "To a certain extent, I can understand that. But that's a good way to keep going the way things are."

Dorrestein definitely wants to change the way things are, particularly for the blossoming field of microbiome research. He views the discipline as passing through phases: the first has centred on determining the identity of microbes. The second phase is working out what they're doing, using techniques such as mass spectrometry.

What drives the establishment of these communities? What metabolic processes are under way, and how do they interact with each other and with a host? "If you fundamentally understand that," Dorrestein says, "you can start to take control of it." And that's the third phase, he says — taking control. By monitoring microbial communities, is it possible to add the necessary ingredients to change a person's health, their mood, their athletic performance? Dorrestein thinks that the answers to these questions are right in front of him. He just has to look a little closer. ■

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