Identifying the elephant

Chapter 7 VALORIZATION

Defining the overall impact of a work like this thesis is a difficult undertaking. As a whole, science is an interconnected web of ideas, experiments, and collaborations, advancing in 1,000 different directions simultaneously. Combining this general trend within the sciences with the ever-increasing drive towards complete omics awareness in biological systems, it becomes obvious that no one scientist can cover every avenue in understanding a biological question. This work has pushed forward that envelope by combining technological innovation in-house with biological questions from abroad, attempting to answer their specific questions.

Scientific Impact

Perhaps the easiest effect to measure from this thesis is the number of collaborative efforts that have spun out from it. Ultra-high mass resolving power combined with mass spectrometry imaging (Chapter 2) lead directly to the National High Magnetic Field Laboratory (Tallahassee, USA) to purchase the same MALDI source for their instruments. This greatly expands the capabilities of that institute and enables many more people the opportunity to explore isobaric compounds in general, as well as the chance to expand further in the capabilities of that system, either in ultraviolet photodissociation or other MS/MS methodologies. As the MagLab is a national laboratory in the United States that all scientists can have access to, this is a wonderful step forward for many people to employ techniques with machinery they otherwise would not.

Building upon the utility of MALDI-2, both in its ability to increase spectral information and its power to image smaller pixels has seen utility in understanding more of the causes in multiple sclerosis. Initial work with the University of Hasselt (Hasselt, Belgium) exposed the presence of Stearoyl-CoA desaturase-1 in lesions of MS. Further information was unveiled in the process of Chapter 3, which has prompted the University of Hasselt to deepen its ties to M4I to further explore the unusual lipidomic alterations unveiled by post-ionization and single cell imaging. Similarly, the Institute of Bioengineering, Ecole Polytechnique federale de Lausanne (Switzerland) utilized this technology to investigate single cell lipidomics in fibroblasts. The Technical University of Munich has developed an on-going collaboration with M4I centered around the understanding of nonalcoholic steatohepatitis in both preclinical mouse models and patient tissue, which is enhanced by the use of MALDI-2 and has unveiled not just fatty acid

changes but specific localization of apoptosis byproducts in the liver. Both the School for Mental Health and Neuroscience (Maastricht, Netherlands) and the University of Gothenburg (Gothenburg, Sweden) have looked to M4I to continue developing our understanding of Alzheimer's disease, and the dyslipidemia that associates with the plaques that are a hallmark of Alzheimer's. Multiple sclerosis, Alzheimer's, nonalcoholic steatohepatitis, these diseases are merely the first set of projects that can be improved by the application of MALDI-2, and the expansion of that technology to new instruments and coupled with different capabilities will only see ever finer understanding of biology.

Shifting away from MALDI-2, the combination of OzID with MSI has shown great potential in studying prostate cancer. The Queensland University of Technology (Brisbane, Australia) has partnered with M4I for the ability to study the global context of cancer progression in human tissue. CID/OzID imaging has already been demonstrated before, but the more difficult double bond isomers have yet to be studied in great depth. However, as demonstrated in Chapters 4 and 5, it is now possible to study these in not just the commonly investigated phosphatidylcholines, but many more phospholipids, as well. As all of the lipid classes are interconnected, but operate with different synthetic pathways, the ability to analyze all of them deepens our ability to understand the whole state of a biological tissue.

Commercial Impact

Through the duration of this thesis, I had the privilege of working with several companies in the pursuit of improving mass spectrometry imaging. Bruker Daltonics (Bremen, Germany), Molecular Horizons, S.R.L. (Brettona, Italy), and Spectroglyph, LLC. (Kennewick, USA) all provided unique opportunities to extend the principles developed in this thesis to wider audiences. Working with Bruker enabled me to apply the expertise I have gained in custom software and hardware packages to a format that is applicable to the routine user, including MALDI-2 and, potentially, data-dependent acquisitions. These data-dependent acquisitions can be read through the auspices of Molecular Horizons' LipostarMSI, a software package that I had the great joy of helping design. Being a vendor-neutral platform, LipostarMSI is useful to scientists working with any commercial instrument and can interpret the complex data derived from data-dependent acquisition experiments (*a la* Chapter 3). Significant use of Spectroglyph's MALDI ion source

for Thermo instruments has also helped refine both physical and software features available from that company.

My contribution to many of these commercial endeavors was as an end user. By offering active feedback on these systems, I have helped make stronger, more robust systems that provide stable platforms for further research. In turn, these companies have helped show me how to constrain the enthusiasm of research to the restrictions of feasibility.

Societal Impact

Perhaps the greatest showing for the societal impact of this work is in the generation of a magazine article about a scientific paper that this work helped publish. The Nederlandse Vereniging van bioMedisch Laboratoriummedewerkers (NVML) asked for an article to be written based on the *Nature* published article "Auto-aggressive CXCR6+ CD8 T cells cause liver immune pathology in NASH," to translate the findings of that work for medical clinicians. That such a magazine article would be requested immediately after the publishing of that journal article points to how impactful this type of research can be, and how necessary it is to make this accessible to more people.

The desire to understand the complete spatial context of tissues is something that will resonate for decades, in my opinion. This work is simply a driving force in making mass spectrometry imaging a vital portion of spatial omics. By providing techniques that can be applied to old systems, programs that can be applied in a vendor-neutral fashion, and showing the utility of combining extant systems, it is my fervent desire that many more people will be able to benefit.