Three-dimensional mass spectrometry imaging of biomedical tissues

Chapter Five

Valorization

Biological tissues are molecularly complex volumetric systems. To understand these tissues techniques are needed that can capture this complexity while maintaining the spatial information. This demands innovative new ways to increase dimensionality, both molecular and spatial, of existing techniques.

Increasing dimensionality is not only needed for research to gain a deeper understanding of complex living systems, in the industry this increase is needed to provide a better diagnosis to patients. A complete tissue gives a better view of the spread of the disease and its severity based on the molecular information. The work presented in this thesis formed a central part of the ITEA 3D Pathology project where the aim was to determine if we can valorize this increased dimensionality by moving 3D pathology to the clinic. In this light, we set out to not only investigate the potential of moving from 2D H&E, which is the standard cell-based analysis used in pathology, to 3D H&E but also the addition of molecular information using 3D-MSI. For this purpose, the consortium consisted of a variety of members focusing on either acquiring the data, data analysis, visualization of the data, as well as setting up a platform to teach pathologists in training how to work and interpret 3D data. All these aspects are of importance and require the collaboration of scientists, companies, and pathologists if 3D pathology is to be established in the clinic. This is how the impact of MSI can be increased beyond the fundamental insights it can provide and provides the possibility to valorize the research described in this thesis.

The scope of this thesis was to investigate the 3D capabilities of MSI and its potential for use in the clinic. Several steps have been added to the valorization potential of this work. The first important step towards clinical translation is to increase the compatibility of MSI with clinical tissues. These tissues are often FFPE tissues that require new sample preparation methods to be developed to allow molecules to be imaged that are still reflective of the biology. Preferably, these sample preparations not only retain the biological information but are also fast to conform to the standard pathology times of analyzing tissues and provide a diagnosis to the patient. In **chapter 2** we, therefore, investigated the effect formalin fixation has on the lipidome of tissues. These results will aid clinicians in better understanding the lipid data acquired from formalin-fixed tissues.

The next phase towards the translation of MSI to the clinic is having established data analysis methods that allow a uniform analysis of the data across different clinics. This is needed to prevent a patient from obtaining diagnosis X at clinic A while with the same data gets diagnosis Y at clinic B. Within M4I we therefore set-out to develop strategies to manage multi-patient data that are presented in **chapter 3**. Our proposed methods ensure that the data used for analysis are without outliers. This is important in a clinic as outliers can steer model-based diagnosis into a false direction thereby reducing the accuracy of diagnosis using this type of data. In addition, the pipeline can be easily transferred to other types of 3D-MSI data sets acquired at different labs. As it is also applicable to single patient 3D-MSI studies or even multi-patient 2D-MSI studies, it makes an ideal starting point towards a uniform data analysis pipeline for clinical 3D-MSI data. This universal application of our method, which is demonstrated in **chapter 4**, is not only interesting in a clinical setting but also for companies when they are considering providing 3D-MSI services.

The previously mentioned aspects are all important to translate 3D-MSI to the clinic. Its true implementation, however, requires integration with the pathologist standard method, which is H&E staining. Within our consortium, we have closely interacted with several pathologists and they also stated that the only way they envision 3D-MSI in their labs is when it can be integrated with their H&E's placed next to it and co-registered to allow regions of interest to be quickly defined.

In view of the above, we collaborated with our consortium partners at Phillips to visualize our MSI data together with the H&E data in their digital pathology system. This was successful and allows both data to be investigated at the same time, however, the data is visualized as single sections instead of a complete 3D volume. As the goal was to integrate both modalities as 3D volumes, we additionally collaborated with PS-Medtech who was also a part of the ITEA 3D Pathology consortium. They provide 3D visualization workstations that are already implemented in hospitals to visualize and interact with 3D CT and MRI data. This interaction is an added value as it will allow the radiologist to explore the 3D image and see what changes. We first set-out to read MSI data into their workstation and

interactive visualize it. Once this was in place we worked towards the end goal of the ITEA 3D pathology, namely visualize a 3D H&E volume next to a 3D MSI volume for the clinical pathology community. This was challenging as two different modalities had to be placed into one coordinate system. In addition, they have to be in the same orientation and coordinate system to ensure the H&E section and the MSI section are the same ones. If these challenges could be overcome we would have everything in place to bring 3D Pathology, and thus 3D-MSI, to the clinic. To this end, a 3D H&E volume was generated at the AMC and with M4I we provided the corresponding 3D-MSI volume. At the end of the ITEA project, we were able to visualize both volumes in one space and interactively go through both volumes simultaneously.

This shows that 3D Pathology can be translated to the clinic but it requires interdisciplinary cooperation of scientists, pathologists, and companies. Only when they all work together across the boundaries of their own disciplines and interests, 3D-MSI analysis can become applicable in clinical diagnostic and provided to pathologists to improve their diagnostic toolbox.