

# Real-Time Molecular Patterns to support Intraoperative Decision-Making

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Chapter 6

# Valorisation

### **The surgical resection and the reference**

Cancer is a leading cause of human death worldwide (1). The treatment of patients with cancer has tremendous repercussions on their quality of life and oncologic surgery is one of the most critical steps of the treatment. Surgeons aim to remove the full tumour from the patient's body while sparing healthy tissue to maintain functions. Intraoperative decisions on the execution of the surgical resection are critical for patient outcome (9-11) and unsuccessful surgeries can lead to tumour recurrence, and therefore, patients may have to undergo reoperation or heavy complementary treatments.

One of the intraoperative challenges is the macroscopic recognition and localisation of tumour tissues by the surgeons. Preoperative, perioperative and postoperative guidance modalities can assist surgeons to localise the tumour in the tissues (12,16,17). Among these modalities, histopathology, the reference for the diagnostic of tumour tissues, can assist surgeons during and after the operation.

Histopathology examination required a multi-step tissue process. Laboratory technicians have to prepare the tissue specimen for histology with fixation, sectioning and staining and then, trained pathologists have to recognize histological features indicative of the disease of the tissue under a microscope. This involves a substantial infrastructure with a laboratory to process tissues, professional experts specialised in their own their pathology discipline. Nevertheless, the subjective interpretation of the tissue sections can lead to divergence in diagnostics between experts.

For perioperative histopathology examination, the tissue specimen is frozen (e. g. in liquid nitrogen) due to the time constraint requested feedback. Pathology examination of frozen sections can be a difficulty due to the quality of the tissue fixation that alters the histological features. For postoperative histopathology examination, the full surgically resected specimen is sent for pathology examination. Tissue is fixed by immersion in formalin for hours due to the rate of penetration of formalin in the tissue. The pathologist selects parts of the tissue for microscopic examination and elaborates a report to provide feedback about the success of the surgery. The elaboration of the report can about a week.

There is a need to assist surgeons for more precise, more reliable surgical executions with providing fast assessment of the disease of the tissue during the surgery.

### **The challenger vs. the reference**

Rapid evaporative ionization mass spectrometry (REIMS), a new ionization source that enables the recognition of tissues based on lipid patterns was employed in the investigation presented in this thesis. Adapted to the classic surgical tools, REIMS can analyse directly the composition of surgical vapours and aerosols generated during surgery and provides feedback in seconds. While current perioperative pathology assessment requires dedicated instruments and trained professionals for tissue processing and examination, REIMS could provide an on-line, real-time pathology assessment while the surgeon is performing the

surgery. The benefit of the REIMS technology for intraoperative tissue recognition appears very appealing to improve surgical precision.

As for any new diagnostic test, a comparison to the reference diagnostic is required to show the accuracy of its performance. Therefore, all the human tissues analysed with REIMS were also subsequently processed for optimal histology and pathology validation to prove the reliability of the REIMS technology for pathology assessment. The accuracy of tissue classifications based on lipid patterns generated by REIMS were compared to the tissue type assessed by the pathologist. The models reached accuracy above 90% accuracy for the distinction of normal and tumour tissues, which prove the performance of the technology.

### **Get in**

Resected tumour tissues from patients undergoing surgery at Maastricht University Medical Centre were collected during the pathology examination of the resected specimen. Right after, tissue slices were directly analysed by REIMS in the laboratory. A library of molecular patterns was established with pathology validation based on these *ex vivo* analyses. The generated models could recognize tissue in the same *ex vivo* context. However, a change of metabolic activity could influence substantially the molecular pattern between *in vivo* and *ex vivo* tissue analyses. Nevertheless, this model generated *ex vivo* enabled recognition of tissue types during breast conserving-surgery showing the proof that an *ex vivo* generated model can recognize *in vivo* tissue types (chapter 4). The translation from *ex vivo* to *in vivo* is of particular interest regarding the previous accomplishments performed by mass spectrometry imaging on clinical studies (chapter 2).

### **Beyond the reference**

The goal of the implementation of the REIMS technology in surgical environments is to assist surgeons with tumour resection for more precise surgery. REIMS classification results were validated by histopathology, the reference for tumour diagnostics. Interestingly, the results generated by REIMS and DESI-MS also showed a change of molecular patterns between tumour border stroma and remote tumour stroma in the application on breast pathology (chapter 4). These results suggest that beyond the reference histopathology validated margin of resection, a molecular margin may exist and could be used to guide even more precisely surgeons. This constitutes a real step forwards to prove the relevance of molecular patterns for surgical guidance.

### **Beyond the surgical resection**

The real-time analysis represents a significant novelty for the recognition of tissue types based on molecular patterns. Mass spectrometry imaging (MSI) has classified tissue types from frozen or formalin fixed samples for years. Molecular patterns based on small metabolites, lipids, trypsin-digested peptides, glycans or proteins enabled relevant classifications for clinical applications such as tumour typing, treatment response and

overall survival (chapter 2). The fact that molecular patterns can be reached in real-time and even *in vivo* questions on how relevant the extracted information from these rapid analyses can be. Precision medicine can benefit from fast tumour metabolic phenotype identification.

Tumour heterogeneity is one of the biggest hassles of cancer treatment. Cancer development is a multistep process stimulated by oncogenic events specific to each patient due to their individual hereditary background and their individual environmental exposure. Moreover, tumour can develop multiple clones each presenting specific biological abilities to metastasis or response to treatment. These inter- and intratumour heterogeneity could be depicted in their metabolic phenotype.

The classification of liver tumours and tumour necrosis based on lipid patterns was studied (chapter 5). It showed that the performance and that the heterogeneity of the lipid patterns could be associated to histopathological changes. Patient specific tumour patterns could be generated to help decision-making. Moreover, a common lipid pattern to discriminate necrosis from viable parts in multiple human tumours was reported. The main discriminators of this necrosis patterns were ceramides and relative intermediates, well-known bioactive lipids for the study of cell death. Targeted analyses on the necrosis patterns based on metabolic shifts led to new perspectives for tumour classification. This investigation showed that real-time molecular patterns can deal with other clinical applications than the only the surgical resection such as tumour heterogeneity. These findings indicate that lipid patterns may have the potential to guide clinical interventions such as drug therapy based on tumour metabolism.

### **Innovative reproducibility**

Mass spectrometry started classifying tissues based on molecular patterns 20 years ago. The field has been driven by technological improvements. The consideration for the biological applications has been limited by the non-existence of reproducibility studies in an MSI field where optimisation of sample preparation and acquisitions are study-dependant. The emerging clinical applications led to the first multicentre studies on breast cancer, one of the most prevalent cancers worldwide (chapter 2).

This thesis includes the work on the first multicentre study on the reproducibility of REIMS for the classification of biological tissues (chapter 3). This study included breast tissues with histopathology validation. REIMS does not require sample preparation, which is an advantage for comparative studies. Moreover, a common methodology was established and key parameters were identified for fair comparison of the library of each site. Quality controls were included on each site to confirm the similar performance of the instruments and repeatability measurements for food items. This study constitutes a major advance in the applications of mass patterns towards their implementations in clinical practice.

### Beyond research purpose only

The investigations reported in this thesis contribute to bring closer to the operating room the molecular patterns-based tissue classification to support decision-making and enable more precise surgical resections. One of the most atypical achievement of this work was the record of electrosurgical vapours *in vivo* during cancer surgeries. These experiments performed on a mobile mass spectrometer required a specific organization with a dedicated patient consent form to participate to the research study and the respect of a safety protocol to fit the risk assessment in place in operating rooms. Information collected from these experiments remained for research purpose only, surgeons were not allowed to adapt their resection to it. Substantial steps remain before the potential use of such technology, including the assessment of its clinical benefit.

The diagnostic use of REIMS combined with the surgical tools based on the analysis of *in vivo* electrosurgical vapours represents a new paradigm in the field of diagnostics devices, and therefore a challenging regulatory framework. It does neither constitute a classic *in vivo* diagnostic test (e. g. administration of drug, isotope complemented with imaging modalities) or an *ex vivo* diagnostic test (performed on materialized biological samples taken from the human body). However, *ex vivo* testing of tissue constitutes an *in vitro* diagnostic test and appears convenient as a first approach to reach its regulation with European and American agencies (European Medical Agency and Food and Drug Administration).

The hospital framework with *in vivo* measurements, the very heterogeneous tissue pathologies and the establishment of tissue libraries with detailed histopathology validation for each tissue sampling are among the challenges of the surgical application. In addition, the advantage of this technology needs to be proven beneficial for patient's outcome with prospective clinical trials which may delay research progress towards diagnostics use of real-time tissue classifiers for histopathology assessment.

The surgical application of real-time tissue classifiers has represented and is still one of the strongest and exciting drive of the mass spectrometric classifiers over the recent years. The investigation of cancer physiopathology with *in situ* molecular patterns to translate gold standard histopathology diagnostics in real-time to improve surgical margin resections constitutes a clinical research field of high interest, and benefit from a clear message to be communicate to a general audience. Nevertheless, on short term, other applications appear already more rentable that the surgical application, with the benefice of robust comparative diagnostics tests and more easily built lipid patterns libraries using high-throughput robotic platform. It is the case for the classifications of microorganisms (293-296) and food items (288-292), and therefore, microbiology and food fraud represent more convenient applications to show the reliability of the technology and reach more accessible markets. These applications may drive the improvement of the technology with high-scale comparative studies over the coming years, and benefit to the development of the surgical application.