Identifying the margin: a new method to distinguish between cancerous and noncancerous tissue during surgery

“There is ... an obvious unmet need for real-time, *in situ* identification of arbitrary tissue features during surgical, therapeutic and some diagnostic interventions.”

Zoltan Takats^{*1}, Julia Denes¹ & James Kinross²

¹Institute of Inorganic & Analytical Chemistry, Justus Liebig University, Schubertstrasse 60, Haus 16, 35392 Giessen, Germany

²Section of Biosurgery & Surgical Technology, Department of Surgery & Cancer, Faculty of Medicine, Imperial College London, 10th Floor, QEQM, St Mary's Hospital, London W2 1NY, UK

*Author for correspondence: zoltan.takats@anorg.chemie.uni-giessen.de

The identification of tumor margins is crucially important in surgical oncology for ensuring a curative resection, accurate prognostication and for sparing of healthy tissues. This latter factor is particularly important in case of neurosurgery, where removal of cubic millimeters of brain tissue from eloquent areas could result in complete loss of psychomotor functions. Currently, tumor margins are established by means of preoperative medical imaging [1–3], and tumors are excised with a predefined safety zone or ‘resection margin’, which is defined by the anatomical location and the diagnosis of the primary tumor. Despite this approach, nearly 30% of breast cancers and 12% of colorectal cancers have an endangered resection margin. In other cases, where the tumor is approaching critically important anatomical features, such as major blood vessels or nerves, more marginal safety zones are used. (By contrast to general surgical approaches, neurosurgery does not apply a safety zone.) Perhaps more importantly, imaging techniques, such as CT scans or MRI, may understage tumors, leaving the surgeon to excise previously unrecognized advanced disease, and operative decisions are made without anatomical data on the disease state [4]. Staging laparoscopy has, therefore, become common place in gastric [5] and oesophageal surgery, and cytology must be used to define peritoneal metastasis [6]. Furthermore, in the emergency setting, imaging techniques may poorly define the anatomy, and often preoperative biopsy data are not available to the surgeon. Finally, the principles of

surgical oncology state that the tumor should not be incised or even handled so as to minimize the risk of metastasis; therefore, the surgeon may not even visualize the tumor itself during the course of the dissection. In the case of the excision of *in situ* cancers (e.g., anal intraepithelial neoplasia), the surgeon will be completely unaware of the tumor boundaries and will, in effect, perform a blind oncological excision.

“...the chemical composition of the tissues follows their histological categorization...”

As a result, more and more intraoperative medical imaging, including MRI, computer-assisted tomography, PET or ultrasonography is being used. Orientation using imaging is facilitated by so-called computer-assisted surgical (CAS) techniques, especially in the case of brain surgery, where it is generally termed ‘neuronavigation’ [7,8]. The first step of a CAS procedure is to create the 3D virtual model of the patient, which is used for planning the intervention. During the actual surgery, a navigation device that determines the exact position of surgical tools on the virtual model, serves as a local GPS for the surgeon. Since there are minimal geometrical changes in the case of the brain and skull (there is a little deformation occurring when cerebrospinal fluid is drained), the system works efficiently. However, in the case of other

Keywords

■ brain tumor ■ cancer surgery
■ intraoperative surgery
■ mass spectrometry ■ rapid evaporative ionization mass spectrometry

surgical specialities, for example hepatobiliary surgery, tissue deformity during surgery causes significant fluctuations in anatomical geometry. Although this can be compensated for by image processing methods, these developments are still in an experimental phase [9]. Nevertheless, an intrinsic problem of the CAS approach is that it cannot compensate for the structural changes caused by the surgical intervention itself, which results in diminishing accuracy throughout the intervention. Since imaging methods do not provide histological-level identification, and cannot usually detect few (10–100) tumor cells in the healthy tissue matrix [10], alternative methods are needed to establish whether the resection of tumor was complete or not. The gold-standard solution for this problem has traditionally been frozen-section histology, which provides biological data for the surgeon [11]. Briefly, the technique involves sending various types of tissue samples to the histopathology lab in flash-frozen format, where the sample is sectioned and stained without proper embedding processes. The resulting sections are investigated by optical microscopy and results are reported, usually via telephone, back to the operating room. Often the resected tumor is sent for frozen-section histology in order to check the entire surface for the presence of tumor cells, while in other cases (especially in the case of breast cancer), sentinel lymph nodes are removed and sent for histopathological assessment. While frozen-section histology results are supported by a knowledge base of almost 100 years, the method still suffers from a number of shortcomings [12]. The most critical point is the nature of analysis, including sampling, transfer of sample to a distant histopathology lab, labor-intensive sample processing, investigator-dependent analysis of the resulting sections and verbal communication of the results. This also has a high financial cost. Sample collection and transfer also raises limitations on the number of sampling points, while the complexity of the procedure results in turnaround times of at least 20 min, with a mean turnaround time of 30–40 min, considerably lengthening the exposure of the patient to a general anesthetic and operative risk. Finally, this approach is dependent on verbal reporting of the specimens, making robust and exact localization of the histological findings on the surgical area challenging.

There is, therefore, an obvious unmet need for real-time, *in situ* identification of arbitrary tissue features during surgical, therapeutic and some diagnostic interventions. This need has been recognized several decades ago; however,

solutions have only been provided in particular cases, mainly in the field of neurosurgery. One innovative solution is certainly the fluorescent labeling of tissues widely used in cases of high-grade astrocytomas, especially glioblastoma multiforme [13]. Labeling is implemented by administration of either the label or its precursor to the patient prior to interventions, which results in the accumulation of fluorescent dyes in tumor tissues.

Recently, chemical characterization of tissues by spectroscopic techniques has been suggested as a general solution to the problem outlined above. Three decades ago, it was recognized that the chemical composition of the tissues follows their histological categorization with surprising correctness [14,15]. Unfortunately, the originally described nuclear magnetic resonance (NMR) spectroscopic methods did not reach the feasible level of routine utilization due to the then low sensitivity of the method and the associated cost of NMR instrumentation. Although the information has become common knowledge, it had not been utilized until the advent of a completely different analytical approach in the late 1990s. Matrix-assisted laser desorption ionization (MALDI) was developed in the late 1980s as an advanced desorption ionization method for the mass spectrometric investigation of nonvolatile compounds and surface analysis of samples [16]. The MALDI technique was first used for spatially resolved analysis of histological tissue sections from the late 1990s (so-called MALDI imaging), and it was immediately recognized that the spectroscopic information follows histology, in a similar fashion to NMR spectroscopy. MALDI imaging provides information on tissue sections that is in complete agreement with the histological analysis of samples; furthermore, the underlying mass spectrometric information is user-independent, unlike the morphology-based classical histopathological approaches [17]. Mass spectrometric (and other spectroscopic) information is objective, in regard to its independence from the personnel performing the analysis, and – although it is rarely pointed out – this means a qualitative difference in comparison with classical histopathology. Nevertheless, MALDI imaging, together with other means of imaging mass spectrometry (including secondary ion mass spectrometry or the more recently developed desorption electrospray ionization) [18], do not go beyond frozen-section histology regarding the complexity of analysis and fall seriously behind regarding the time demand. Imaging mass spectrometric studies prove that mass spectrometric

profiling information can be used for the unambiguous identification of tissues; however, until very recently, no mass spectrometric ionization technique was able to provide the required data *in situ*, in order to present a competitive alternative to histopathology.

“...online mass spectrometric information can be translated to histology-level identification of tissues on the timescale of a single second.”

Rapid evaporative ionization mass spectrometry was exclusively developed for intraoperative mass spectrometric investigation of tissues *in vivo* [19–21]. The technique is based on the discovery that surgical dissection techniques, such as electrosurgery or laser surgery, also act as an ionization method, such as converting molecular components of vital biological tissues into gaseous ions amenable to direct mass spectrometric analysis. The resulting data – featuring mainly complex lipid-type species – is highly similar to that provided by imaging mass spectrometry; hence, it is also tissue-specific. Combination of surgical tools with online mass spectrometric analyses resulted in the concept of so-called ‘intelligent knife’; a fully functional surgical cutting tool, which also analyses the dissected tissue parts. Tissue identification by the means of the rapid evaporative ionization mass spectrometry technique is based on comparison with authentic spectra, using multivariate statistical pattern recognition methods, including principal component analysis and linear discriminant analysis. As a result, online mass spectrometric information can be translated to histology-level identification of tissues on the timescale of a single second. Since then, while the intelligent knife concept was originally developed for electrosurgery [19], alternative surgical techniques (e.g., laser surgery and ultrasonic dissection [21,22]) were also successfully coupled with mass spectrometric analysis. The intelligent knife has the potential to revolutionize the identification of tumor margins in two fundamental ways: first, it can deploy an ‘alerting mode’ for the surgeon during the tumor resection and when working close to the solid tumor tissue. Whenever the tumor tissue is approached, the device gives alerts to the surgeon to lead the resection line further from the bulk tumor tissue. Infiltrations and proximal metastases induce positive tissue identification results, warning the surgeon of the presence of tumor cells in the resection line. This approach could potentially guarantee

clear margins and, in principle, would result in minimized removal of healthy tissue. Mass spectrometric chemical profiling also allows the detection of tumor environment without actually cutting into the bulk tumor tissue, hence the risk of metastasis formation is not increased significantly by this approach. Second, the intelligent knife may be used in the so-called ‘microprobe mode’, where a miniaturized probe (not necessarily a surgical tool) is used for the evaporation (or other disintegrative sampling) of minute amounts of tissue. rapid evaporative ionization mass spectrometry technology enables the identification of as little as 50 µg of tissue material, so the sampling is minimally invasive. In this case, the surgeon, endoscopist, radiologist or therapeutic technician can sample any suspicious tissue feature on the surgical area and get histology-level identification within a single second.

Overall, there has been considerable technical advancement regarding the identification of tumor margins during the last few decades. While histopathology is gradually moving towards the detection of genetic and expression markers for personalized medicine and patient group stratification, *in vivo* tissue identification is increasingly becoming the task of medical imaging, in combination with spectroscopic methods. NMR spectroscopy, also known as magnetic resonance spectroscopy, has the advantage of including both modalities; however, NMR spectroscopy still cannot provide the type of data necessary for unambiguous tissue identification in real-time. By contrast, although mass spectrometry is capable of tissue identification *in situ* in real-time, it is not yet capable of *in vivo* imaging. One can envision these two technologies symbiotically working together in the future for accurate tumor margin identification, most likely cemented together by the framework of CAS.

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