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Intraoperative tissue identification by mass spectrometric technologies

Edward R. St John [a], Merja Rossi [b], Pamela Pruski [b], Ara Darzi [a], Zoltan Takats [b,*]

[a] Department of BioSurgery and Surgical Technology, Imperial College London, London W2 1NY, UK
[b] Division of Computational and Systems Medicine, Imperial College London, London, SW7 2AZ, UK

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ABSTRACT

Mass spectrometric (MS) approaches developed for tissue identification in surgical environments are reviewed. MS Imaging (MSI) techniques enable the direct analysis of human tissue and can be used as an alternative means for margin assessment. While MSI-based approaches were demonstrated to improve the examiner-related variance of the data, the time demand and the cost of these analyses remained high. Furthermore, the necessity of MS expertise for the clinical deployment of these techniques has hindered large-scale clinical testing. The advent of 'ambient' MS methods contributed to the application of MSI techniques in this field, however alternative methods have been developed for the direct analysis of tissue samples without sample preparation. One group of methods employs surgical tissue manipulation for ionization while the other one uses minimally invasive probes for sampling prior to ionization. The methods are summarised and compared with regard to the information delivered, turnaround time and tissue identification performance.

Contents

1. Introduction

The presence of cancer cells on the edge of the surgical resection margins (positive margins) is usually confirmed by postoperative histopathological examination of the resected tissue with results only available after the surgery has finished. Nevertheless, histopathological examination is also available during the surgical procedure, however its reliability is inferior compared to that of postoperative approaches. Traditional histopathological techniques to provide a tissue diagnosis during surgery include frozen section and imprint cytology. Frozen section analysis involves rapid freezing, cutting, staining and examination of a section of tissue. Imprint cytology involves touching excised tissue or fluid to a slide and both are based on microscopic assessment by a highly skilled pathologist. These techniques for intraoperative margin assessment (summarized in Table 1) are time consuming, laborious and

Abbreviations: 2-HG, 2-hydroxyglutarate; CBS-MS, coated blade spray mass spectrometry; CT, computed tomography; CUSA, Cavitron ultrasonic surgical aspirator; DESI, desorption electrospray ionization; DESI-MS, desorption electrospray ionization mass spectrometry; DESI-MSI, desorption electrospray ionization mass spectrometry imaging; IDH1, isocitrate dehydrogenase 1; LC-MS, liquid chromatrography mass spectrometry; LDI-MS, laser desorption ionization-mass spectrometry; MALDI, matrix-assisted laser desorption/ionization; MALDI-MS, matrix-assisted laser desorption/ionization mass spectrometry; MALDI-MSI, matrix-assisted laser desorption/ionization mass spectrometry imaging; MS, mass spectrometry or mass spectrometric; MSI, mass spectrometry/spectrometric imaging; PESI-MS, probe electrospray ionization mass spectrometry; PS-MS, paper spray mass spectrometry; REIMS, rapid evaporative ionization mass spectrometry; RF, radio frequency; SESI, secondary electrospray ionization; SIMS, secondary ion mass spectrometry; SPME, solid phase microextraction; V-EASI, Venturi easy ambient sonic-spray ionization.

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* Corresponding author. Tel.: +44-207-5942760.
E-mail address: z.takats@imperial.ac.uk (Z. Takats).

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Table 1
Intraoperative margin assessment techniques

Technique	Reference	Time required	Sample type (number)	Sensitivity	Specificity
Frozen sections	***[3]***	20–25 min	Breast (gold standard method for most tumor tissue)	73%	98%
Touch imprint cytology	***[4]***	10 min	Breast (used for most tissue types)	89%	92%
Intraoperative ultrasound	***[5]***	3–6 min	Breast (also used for other tissue types)	75%	81%
Mohs micrographic surgery	***[6–8]***		Facial recurrent basal cell carcinoma	59–100%	81–90%
Digital specimen X-ray	***[9]***	1–20 min	Breast (intraoperative specimen mammography)	66%	86%
Micro-computed tomography	***[10]***	7 min	Breast (Shaved cavity margins, 25 samples)	83%	95%
High frequency ultrasound	***[11]***		Breast (Ductal carcinoma, 17 patients)	74%	85%
Diffuse reflection spectroscopy	***[12, 13]***		Breast (Two studies with 47 specimens and 55 specimens)	90%	88%
Raman spectroscopy	***[14, 15]***	3–5 s	Brain and breast tissue	83–93%	91–93%
Optical coherence tomography	***[16]***		Breast	100%	82%
Confocal microscopy	***[17] [18]***		Brain (33 lesions), bladder	79–90%	67–88%
RF impedance spectroscopy	***[19, 20]***		Breast	70–75%	56–70%

Table 1 displays examples of current and emerging intraoperative margin assessment techniques. Legend: RF = Radio-frequency.

subjective. Positive margins are, however, directly linked to a higher rate of local/regional recurrence and require a further operation to remove the remaining cancerous tissue. In breast surgery, where the current trend is to conserve as much healthy tissue as possible, the overall re-operation rate is about 20% or over [1, 2]. Based on the number of recent publications on intraoperative margin identification, assessing margins is most relevant in breast, neurological, urological, upper gastrointestinal, colorectal and skin cancers. Due to the importance of margin assessment, recent research has actively focused on novel margin detection technologies using a variety of analytical techniques in addition to traditional histology. Broadly these can be divided into medical imaging techniques, optical spectroscopy, radiofrequency spectroscopy and mass spectrometry (MS).

Traditional methods for tissue identification are based on detecting morphological changes in different tissue types. In addition to changes in structure and form, cells in different tissues have highly specific chemical composition, which can easily be profiled using mass spectrometric techniques. In the last few years development of novel techniques and instrumentation for mass spectrometry has led to the ability to detect proteins and metabolites directly from tissues using desorption ionization techniques, paving the way to the development of intraoperative mass spectrometric approaches. Currently developed methods fall into three main categories including intraoperative imaging, surgical device-based ionization techniques and solid sampling probes for subsequent MS analysis. The current review follows this structure for the description of the individual approaches.

2. Discussion

2.1. Mass spectrometric imaging

Various mass spectrometry techniques have successfully been used to characterize tumor microenvironment and margin involvement in *ex vivo* samples (Table 2). Many of these are mass spectrometry imaging (MSI) approaches, which use a workflow based on traditional histology, displayed in Fig. 1. In these cases, tissue specimens collected intraoperatively are flash-frozen and sectioned. However, instead of using staining and microscopy to identify the cell types present, the sections are analysed by MSI, obtaining spatially resolved chemical information. The aim of these experiments is to detect certain mass spectrometric peaks or spectral patterns typical for the tumor tissue or tissue infiltrated by cancer cells. In recent years, imaging mass spectrometry has undergone tremendous development with regard to speed of analysis, sensitivity and spatial resolution. Furthermore, the improved performance characteristics enabled conducting large scale, properly powered imaging studies which provided broad evidence for the histological specificity of MSI data, paving the ways for the intraoperative deployment of the technology.

The three most commonly used MSI techniques in tissue imaging are secondary ion mass spectrometry (SIMS), matrix-assisted laser desorption mass spectrometry (MALDI-MS) and desorption electrospray ionization mass spectrometry (DESI-MS). The first of these to emerge was SIMS, which was originally developed for the elemental analysis of structured inorganic surfaces [21, 22] in the 1960s. SIMS uses a focussed high energy primary ion beam consisting of ionized atoms or clusters for the desorption and ionization of species (atoms or molecules) present at the gas/solid interface. Traditionally SIMS has been used for elemental analysis and detection of organic fragments, but more recently larger projectiles consisting of thousands of argon atoms have allowed higher secondary yields and detection of intact molecules up to 2–3 kDa molecular weight [23]. SIMS allows the highest spatial resolution of all current MSI techniques [24] however both the preparation of samples and the analysis itself are complex and time consuming, which raises serious problems regarding the intraoperative applicability of the method [25–27].

In contrast, MALDI has been routinely used for analysis of tumor margins and tumor microenvironment in fresh frozen tumor samples [28, 29]. Although the method requires the careful deposition of matrix compound on the surface of the sample, the overall complexity of the analysis workflow is significantly lower compared to SIMS. In addition, MALDI readily detects proteins, peptides, complex lipids and metabolites in a single experiment as it was described in the case of gastrointestinal [30], respiratory [31], bladder [32], breast [33], and ovarian [34] cancers, as well as in acute myeloid leukemia [35]. Although MALDI has also been considered to be time consuming, the recent commercialization of the Rapiflex instrument by Bruker (Massachusetts, United States) reduced the analysis time of a single sample to a few minutes, opening the door to intraoperative deployment of the technology.

In contrast to SIMS and MALDI, DESI is capable of the analysis of the sample surface under atmospheric pressure and temperature and it requires minimal or no preparation or pre-treatment [36]. The DESI experiment is implemented by directing a pneumatically assisted solvent electrospray onto the surface of interest. The incoming droplets accumulate on the surface extracting all soluble analytes and the impact of further primary droplets produce secondary droplets which contain the dissolved analyte molecules. The secondary charged droplets follow an electrospray-like ionization scenario, yielding mostly electrospray-like spectral data. DESI analysis of tissue sections produces spectra dominated by complex lipid species including phospholipids, triglycerides, sphingolipids and cardiolipins, due to the high concentration and preferred ionization of these species. Nevertheless, the histological specificity of these

Table 2
Mass Spectrometry in biological samples, techniques with potential relevance to Intraoperative Mass Spectrometry

Year/Ref	MS Method	Ion mode (mass range)	Biological sample	Analytes detected	Number of samples (S) or patients (Pt)	Imaging	Time demands of anlalysis
Matrix assisted laser desorption ionization imaging mass spectrometry (MALDI-MS)							
2014 [49]	*MALDI-MS*	+ve	Human lung (homogenized frozen samples)	40 peaks that differentiated cancer and normal tissue	138 Pt and 141 tumour/non-cancer pairs and 8 unique cancer samples	No	Not available
Matrix assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI)							
2013 [50]	*MALDI-MSI*	+ve, (400–1,000)	Human breast (frozen sections)	Lipids	29 Pt and S	Yes	Not available
2014 [51]	*MALDI-MSI*	(50–1,000)	Human colorectal (frozen sections)	Lipids	12 Pt	Yes	Not available
2015 [52]	*MALDI-MSI*	+ve, (500–30,000)	Human pituitary (frozen sections)	Protein/Peptide hormones	51 S	Yes	<30 minutes
Desorption electrospray ionization mass spectrometry (DESI-MS)							
2005 [37]	*DESI-MS*	+ve	Mouse pancreas, rat brain and human liver adenocarcinoma (frozen sections)	Lipids	Animal tissue, a case study of human liver adenocarcinoma	No	Less than 5 seconds per spot
2013 [53]	*DESI-MS*	Not available	Rat brain, kidneys and intestine (frozen sections)	Lipids	Animal tissue	No	0.5 seconds (Immediate analysis in laboratory)
2014 [39]	*DESI-MS*	Depends on analyte	Human brain gliomas (frozen tissue- *ex vivo*, intraoperative)	2-hydroxyglutarate	35 S *ex vivo*, 2 S *in vivo*	No	Within minutes
Desorption electrospray ionization mass spectrometry imaging (DESI-MSI)							
2011 [54]	*DESI-MSI*	-ve, (600–1,000)	Human brain – tumor tissue (*ex vivo*, intraoperative, frozen sections)	Lipids	Brain tumor samples from different locations, one case study presented	Yes	Not available
2012 [46]	*DESI-MSI*	-ve and +ve, (200–1,000)	Human brain - tumor frozen sections (*ex vivo*, frozen sections)	Lipids	55 S *ex vivo*, 32 S / 5 Pt intraoperative	Yes	Not available
2013 [48]	*DESI-MSI*	-ve, (50–1,100)	Human brain – Glioblastoma (*ex vivo*, frozen sections)	Lipids	12 S / 1 Pt	Yes	1 s
2013 [47]	*DESI-MSI*	-ve	Human brain – Oligodendroglioma, astrocytoma, and meningioma tumors (*ex vivo*, frozen sections)	Lipids	32 S / 5 Pt	Yes	Less than a a second
2014 [41]	*DESI-MSI*	-ve, (90–1,200)	Human stomach (frozen sections) *Ex vivo*: cancer and normal gastric tissue *In vivo*: gastric cancer margin	Lipids, small molecules related to energy production	62 S (*ex vivo*), 21 S / 9 Pt (intraoperative)	Yes	0.5 s per pixel
2014 [40]	*DESI-MSI*	-ve, (50–1,100)	Human breast tissue (*ex vivo*, frozen sections)	Lipids	61 S / 14 Pt	Yes	Within seconds
2015 [55]	*DESI-MSI*	-ve (200–1,000)	Human breast (*ex vivo*, frozen sections)	Free fatty acids & Lipids	Lesion = 28 S / 28 Pt Tumour bed = 98 S / 22 Pt	Yes	Not available
Surgical ionization techniques							
2011 [56]	*LDI-MS*	-ve, (150–1,000)	Human colon carcinoma metastasis in the liver, porcine kidney, porcine lung (*ex vivo*)	Lipids	Not available	No	Not available
2011 [57]	*V-EASI*	-ve and +ve, (150-1,000)	Human brain – astrocytomas, meningeomas, metastatic brain tumors and healthy brain tissue and human liver tumor tissue (*ex vivo*)	Lipids	Not available	No	2–3 seconds
2013 [58]	*REIMS*	-ve, (600–900)	Human: gastric, colorectal, liver, breast, lung and brain tissue (*ex vivo* and intraoperative)	Lipids	302 Pt (*ex vivo*), 81 Pt (intraoperative)	No	2.5 seconds
Other ionization techniques							
2011 [59]	*PS-MS*	-ve, (depends on analyte)	Rat brain tissue, porcine adrenal gland, mouse liver tissue, human prostate tumor and normal tissue (*ex vivo*)	Hormones, lipids and therapeutic drugs	Animal tissue, case study of human prostate tumor and normal tissue	No	Less than 1 minute
2012 [60]	*PESI-MS*	Depends on analyte	Human Kidney (Frozen homogenized kidney tissue, intact kidney tissue (*ex vivo*))	Lipids	9 Pt	No	5–15 minutes
2013 [61]	*PESI-MS*	-ve, (depends on analyte)	Human colon tumor tissue (*ex vivo*)	Lipids and proteins	8 S	No	Not available
2014 [62]	*CBS-MS*	Depends on analyte	Phosphate buffered saline spiked with cocaine, human plasma and urine samples	Cocaine and diazepam (drugs)	Phosphate buffered saline, plasma and urine samples	No	Less than 3 minutes
2015 [63]	*SPME-MS*	-ve	Individual small organisms (*Daphnia magna*)	Perfluorooctanesu-lfonic acid and perfluorooctanoic acid	28 S	No	Within minutes
2016 [64]	*SPME-MS*	Depends on analyte	Phosphate buffered saline spiked with cocaine and diazepam	Phosphate buffered saline	Plasma and urine samples = 2S	No	Within minutes

Table 2 details studies with potential application to intraoperative mass spectrometry. Legend: CBS-MS coated blade spray mass spectrometry, CUSA Cavitron ultrasonic surgical aspirator, DESI-MS desorption electrospray ionization mass spectrometry, DESI-MSI desorption electrospray ionization mass spectrometry imaging, MALDI-MS matrix-assisted laser desorption/ionization mass spectrometry, MALDI-MSI matrix-assisted laser desorption/ionization mass spectrometry imaging, REIMS rapid evaporative ionization mass spectrometry, LDI-MS laser desorption ionization-mass spectrometry, V-EASI Venturi easy ambient sonic-spray ionization, PESI-MS probe electrospray ionization mass spectrometry, PS-MS paper spray mass spectrometry, SPME solid phase microextraction, Ref references.

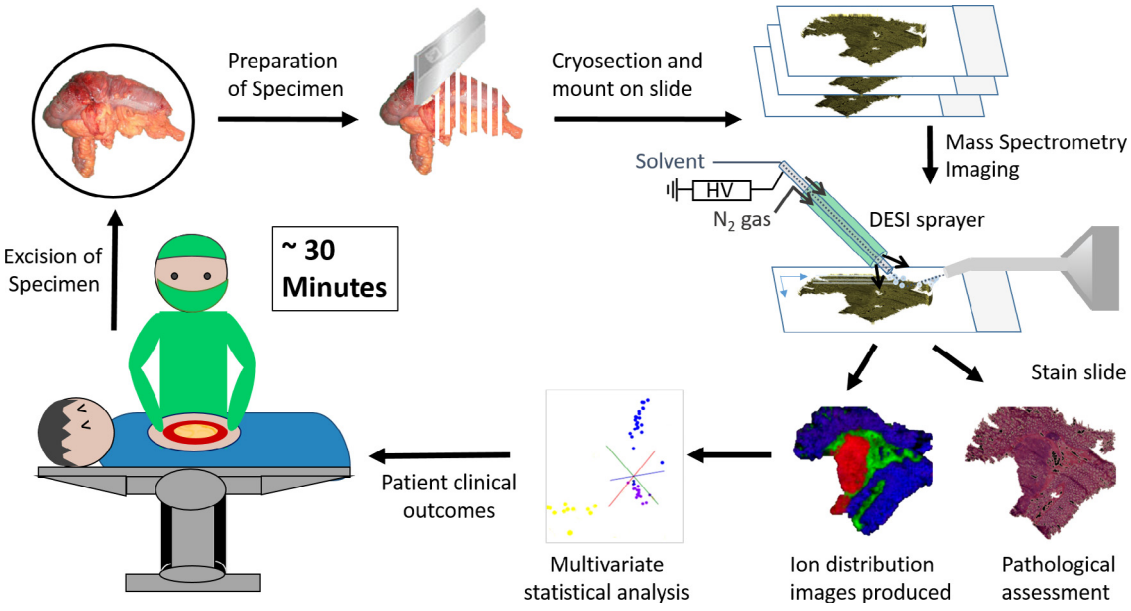

Fig. 1. Mass spectrometric imaging workflow. An optimized MSI workflow is displayed. The process involves surgical excision of the specimen, gross preparation of specimen followed cryosection and mounting on slides. Tissue on slides are analysed by MSI method. After MS analysis slides can be sent for secondary pathological assessment. MS images can be assessed intraoperatively for surgical decision making.

lipid profiles obtained by DESI-MSI has been demonstrated in the case of liver cancer [37], prostate cancer [38], brain tumors [39], breast cancer [40] and gastrointestinal tumors [41]. In one of these studies, DESI-MS was used to measure differences in levels of a polar metabolite, 2-hydroxyglutarate, providing evidence that the technique is capable of detecting non-lipid type species [39]. The metabolite, which is derived from α-ketoglutarate, is known to accumulate in leukemia and specifically in most grade two and three gliomas in adult patients due to a mutation in isocitrate dehydrogenase 1 and 2 and could be used as a biomarker [42, 43]. DESI-MS was able to identify an increase in 2-HG that was independent of the exact mutation of IDH1. This compares well with immunohistology which is only able to recognize certain types of the many IDH1 mutants using a specific antibody (anti-IDH1 R132H [44]). An intraoperative model for the detection of the biomarker was constructed and increased levels of 2-HG were detected from intraoperative samples of glioma containing a mutation in IDH1 in the operating room. Using DESI-MS installed within the operating room, the group was able to detect 2-HG within minutes from intraoperative glioma specimens containing mutant variants of IDH1 [39]. N-acetyl-aspartic acid, detected using DESI-MS in neurological specimen smears, is another recent example of an oncometabolite that has been used to help differentiate between brain parenchyma [45]. Furthermore, this same study demonstrated that multivariate statistical analysis of spectra from a combination of lipids and oncometabolites including 2-HG resulted in accurate differentiation between tumour type (glioma, meningioma and pituitary) with an overall sensitivity of 99.4% and a specificity of 99.7%. These are just a few examples demonstrating how chemical profiles obtained from tissue have potential to offer additional information compared to traditional histology.

In another experiment, statistical analysis of DESI-MS data was able to differentiate between gliomas (oligodendrogliomas, astrocytomas and oligoastrocytomas) representing a variety of grades using lipid species such as fatty acids, glycerophosphoinositols, glycerophoserines, plasmenyl phosphoethanolamines and sulfatides [46]. Classifiers constructed from multivariate statistical analysis of spectral signatures and correlated with histopathology revealed a high cross-validated predictive accuracy of 97% for tumor type, grade and cellular concentration. Models based on lipid profiles have provided similar results when distinguishing gliomas from meningiomas [47], necrotic tumor tissue from viable glioblastoma multiforme [48] and in breast cancer margin assessment [40]. The authors of the studies concluded analysis in the negative ion mode revealed greater sensitivity for the detection of lipids and metabolite species [40, 41, 46, 48].

MSI techniques have provided large quantities of data that is highly relevant to tissue identification in a histological setting and understanding biological processes in cancer-host tissue systems. However, with the exception of tissue smears, the current MSI techniques require preparation of frozen tissue sections prior to analysis. All procedures can lead to spatial dislocation of chemical species or modification of the chemical profile on the tissue and preparation via frozen section adds significantly to the time it takes to analyse a sample, since a single section is rarely representative for the status of the entire surgical margin. This inability to produce results within the timeframe of the operation as well as the requirements arising from sample preparation limit their usefulness for intraoperative margin assessment. Fig. 1 illustrates an optimized MSI workflow.

2.2. *Intraoperative mass spectrometry*

While MSI techniques demonstrate the potential of mass spectrometry for rapid proteomic or lipidomic phenotyping of tissues yielding histologically relevant information, their clinical translation to surgical applications is seriously hindered by the complexity and time demand of these approaches. Nevertheless, on the basis of this potential, a range of ambient ionization techniques better suited for surgical use have been developed (Table 2). These techniques generally utilize surgical tissue manipulation methods for the ionization of cellular component, eliminating the need to introduce novel tools into the surgical area and offering the possibility of in-situ, real-time analysis. The rationale of these methods is based on the consideration that the rapid thermal or mechanical disintegration of aqueous systems (e.g. tissues or biological fluids) produces charged droplets, which can eventually yield gas phase ions upon the evaporation of the aqueous phase. These mechanisms are assumed

to be similar to that of sonic spray or thermospray (off-filament mode) ionization methods. Since most surgical tissue dissection methods (with the obvious exception of surgical scalpels) thermally or mechanically disintegrate tissues, they were also expected to function as ion sources. The development of these techniques for intraoperative tissue identification thus only required the addition of a suction line to the surgical handpieces and providing efficient means for ion/aerosol transfer to a mass spectrometer installed several meters away from the patient. Similarly to the above mentioned sonic spray ionization and thermospray ionization techniques, various surgical techniques including laser surgery, electrosurgery or ultrasonic tissue ablation produce low intensity but still informative mass spectra upon the introduction of the resulting aerosol into a mass spectrometer equipped with an atmospheric interface. The efficiency of ion yield was successfully improved by inducing surface-induced dissociation of aerosol particles in the atmospheric interface of the instrument or by secondary electrospray (SESI) post-ionization. The discovery that various surgical tools incidentally cause ionization of tissues during routine surgical use has been of great significance and these techniques are described below.

Laser desorption ionization-mass spectrometry (LDI-MS) has originally been developed in the 1970's primarily for elemental analysis purposes, and later it was successfully used also for the ionization of organic molecules including components of biological tissues. Instrumentation capable of LDI-MSI was developed in the early 1980's. The advent of MALDI in the late 1980's, however effectively halted the further development of LDI-based tissue analysis and the technique (in the form of laser surgery) was re-discovered around 2010 as a potential platform for intraoperative tissue identification [56]. The employed CO_2 lasers are one of the most common lasers used in a variety of surgical procedures, since they offer precise cutting and coagulation with minimal thermal spread. Recent advancements have heralded the flexible CO_2 laser that enables use in a wider range of applications including brain and endoscopic surgeries [65, 66]. Aerosol is produced as a direct results of laser application on tissue and can be aspirated continuously into a mass spectrometer. Analysis of *ex vivo* samples of human liver colorectal metastasis and human renal cell carcinoma using CO_2 LDI-MS showed good separation between cancer and surrounding healthy tissues using supervised multivariate statistical methods. The technique is not limited to CO_2 lasers, practically any lasers with wavelengths in the 190–580 nm and above 2 μm produces ions on the ablation of biological tissues. The resulting spectral data features ions corresponding to various lipid compounds, similarly to SIMS or DESI. It's important to point out that no photochemical effects or wavelength dependence of spectral patterns were observed, supporting a largely thermal ionization mechanism [56].

Cavitron ultrasonic surgical aspirator (CUSA) has also been successfully coupled with on-line MS analysis of dissected tissue material. The technique uses ultrasound to induce the mechanical disruption of tissues combined with a continuous flushing mechanism to clear away the resultant tissue debris. Primarily used in neurosurgery it works as a precise technique for selectively excising parenchymal tissue whilst sparing blood vessels, it can also be used in operations targeting the liver and pancreas. The technique was coupled with MS by transferring and aerosolizing the tissue debris using a Venturi fluid pump for both functions [57]. The pneumatic spraying of the sample was shown to produce ions corresponding to predominantly complex lipid species. Other small molecules not readily detected by other ambient ionization mass spectrometry techniques using a thermal process were also detected. The technique has been used to analyse *ex vivo* brain and liver samples [57]. A clear difference was seen between spectra corresponding to grey and white brain matter as well as different types of brain tumors. Liver tumors also separated well from healthy liver parenchyma. The analysis was performed in 2–3 seconds from CUSA application, leading to near instant measurements that are significantly faster than the sample preparation for DESI-MS described in a previous paper [54].

The third surgical modality serving as a potential ion source is electrosurgery or electrosurgical diathermy [58]. This technique utilises radiofrequency (>100 kHz) alternating electric current for the dissection of tissues and coagulation of minor blood vessels. The mass spectrometric analysis of electrosurgical aerosols was first described in 2008 and the combination was termed Rapid Evaporative Ionization Mass Spectrometry (REIMS). REIMS is implemented either by directly aspirating electrosurgical aerosol into the MS using the suction force generated by the vacuum system or can also be delivered to the instrument by Venturi pump. REIMS has been extensively tested to date by analysing thousands of *ex vivo* specimens consisting of normal and cancerous tissue. The reports feature healthy and cancerous tissues from stomach, colon, liver, lung and breast samples among others. Spectral data shows intensive signal in the mass range 600–900 representing predominantly lipids, similarly to data produced by many other direct ionization approaches. The sensitivity and specificity of the method was reported to range from 69% for mixed adenosquamous cell carcinoma to 100% for healthy tissue from lung and liver [58]. Further validation was obtained by acquiring *in vivo* data in the operating theatre for 81 patients. The sensitivities and specificities from this analysis ranged between 95–100% and 92–100% respectively. All data points were acquired and analysed within 2.5 seconds, resulting in near real time feedback during surgical intervention. Fig. 2 illustrates an optimized surgical ionization workflow.

2.3. Sampling probe-based methods

A persistent problem encountered in the analysis of biological tissue is access to tissue features without surgically exposing them. For most techniques, a physical sample has to be either exposed or removed from the body prior to MS analysis. A group of MS methods employing needle geometry sampling probes can provide a solution for this problem (Table 2). The sampling needle in this case is introduced into the tissues from a few mm to few cm depth and analysed either directly or following solvent extraction. The simplest implementation of the approach is probe electrospray ionization mass spectrometry (PESI-MS), which uses a small probe or solid needle for sampling and subsequent electrospray ionization [67]. The technique was used for analysis of *ex vivo* samples of human clean renal cell carcinoma: tissue was placed onto a sampling stage and a solid needle was lowered into the sample and then raised out to allow application of high voltage and subsequent electrospray ionization. The cancerous regions in the sample were correctly identified when correlated with histopathology. Interestingly, comparisons between homogenised samples and intact specimens revealed similar spectral profiles suggesting that intracellular lipids could be measured without homogenisation [60]. Following on from this work the same group developed a new method incorporating their PESI-MS design in negative ion mode with an auxiliary side solvent vapour sprayer using isopropanol-H_2O. Principal component analysis showed defined separation of cancerous and normal colon tissues [61]. While capable of rapid identification of sufficient lipid profiles to classify normal and tumor tissue [61] and even analysis of metabolites in single cells [68], these techniques are more suited for analysis of biopsy samples. Fig. 3 illustrates an optimized probe base ionziation workflow.

The sampling probe can also carry an absorbent layer to improve selectivity and sensitivity of the tissue extraction. Solid phase microextraction (SPME) devices are well suited for this purpose and the utilization of SPME technology for intraoperative tissue identification was reported recently. A thin and flexible probe was made with a core built from titanium-nickel alloy and a biocompatible

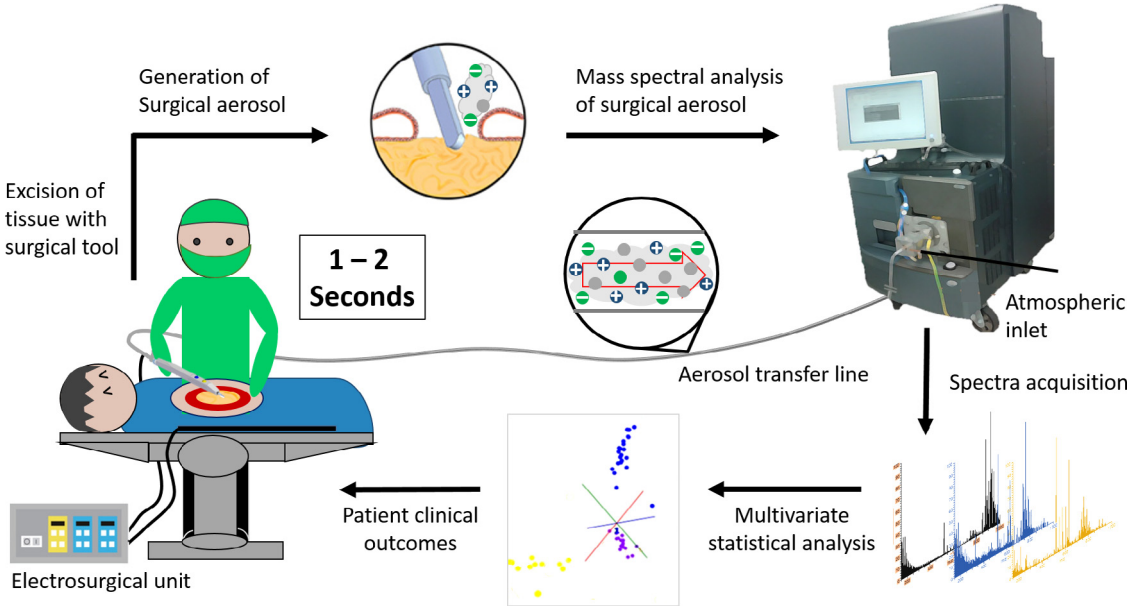

Fig. 2. Surgical ionization workflow. An optimized surgical ionization workflow is displayed. A surgical tool is used as per standard operating procedure. Surgical aerosol is aspirated and delivered to the mass spectrometer. MS analysis is performed, results are interpreted by multivariate statistical models and displayed on screen for immediate feedback to the surgeon.

co-polymer for the extraction substance that coats part of the probe. The probes are directly immersed into the tissues and eluted following the tissue extraction step. The eluates are analysed by LC/MS, however the SPME can also be used similarly to PESI or can be directly analysed by DESI-MS. The technique allows for the extraction of small metabolites and drugs with molecular weights of up to 1000 Da. These probes have been successfully used for the intraoperative analysis of a wide range of metabolites during lung and liver transplantation in porcine models [69]. Levels of endogenous compounds and drugs revealed changes in relation to cold ischemic time, perfusion and reperfusion. Although this technique has not yet been used in *in vivo* human tissue, it is easy to see how this simple technique is readily translatable to human surgical applications and could augment or replace the traditional core biopsy procedure.

An advantage of SPME based analysis is that the coatings can extract both polar and non-polar compounds. This technique is also ideally suited for the extraction of small-molecular-weight analytes which enables detection both of endogenous and pharmacologic molecules [70]. Previously, rapid analysis of the sample from the SPME probe was a challenge. Coated blade spray (CBS) ionization mass spectrometry was developed specifically for the rapid analysis of SPME samples and was demonstrated to improve the speed of analysis to within 3 minutes[62]. A stainless steel blade coated with the biocompatible polymer C_{18}-polyacrylonitrile (C_{18}-PAN) is preconditioned in a methanol and water mix. The blade is then inserted into the sample matrix and agitated with a vortex for 1 minute. Following this the blade is rinsed in water prior to desorption/ionization initiated by the passing a high voltage through the blade. Recently, the same group has coupled SPME fibres with

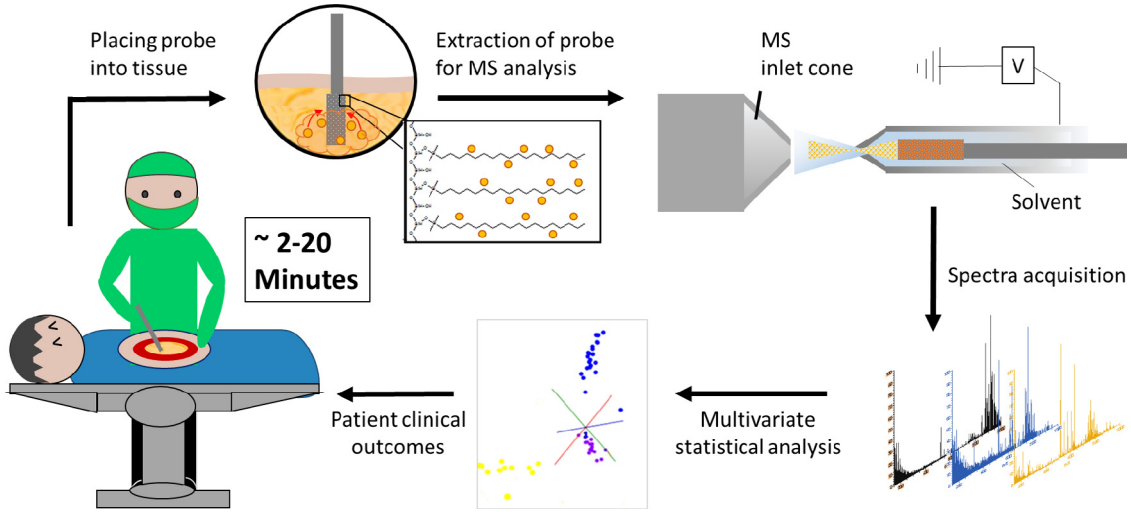

Fig. 3. Probe based ionization workflow. An optimized probe based ionization workflow is displayed. A probe is inserted into the appropriate tissue for approximately 2-20 minutes allowing the diffusion of analytes onto the sampling matrix. The probe is extracted, a solvent added and a voltage applied. Ionized spray is aspirated into the MS for analysis. Mass spectra are acquired for interpretation by multivariate statistics. Results are fed back to the surgeon for intraoperative decision making.

the use of nano-electrospray ionization (nano-ESI) decreasing sample preparation time to 2 minutes [64]. Accurate results are demonstrated for probe analysis in phosphate-buffered saline (PBS), urine, and whole blood. The technique is readily applicable to tissue biopsy or biofluids within the operating room, however, work is also underway to investigate the use of SPME-nano-ESI as a rapid diagnostic technique *in vivo*.

This class of rapid mass spectrometry techniques additionally includes paper spray mass spectrometry (PS-MS) which utilizes a cellulose-based adsorbent and on-probe electrospray. PS-MS process comprises placing the sample onto a triangular strip of porous paper for a few seconds and applying a solvent and a high voltage across the paper inducing an electrospray of charged ions that is aspirated into a mass spectrometer for analysis [59]. The technique was demonstrated to yield histologically specific mass spectral data in less than a minute. Due to the large amount of liquid present in the surgical area the PS method suits better for the analysis of core or pinch biopsy specimens.

3. Conclusion

Mass spectrometric analysis of tissues in the surgical environment is a rapidly developing field. Although there are a number of different technologies which are potentially able to replace the currently used tissue morphology-based approach, mass spectrometry shows a few unique advantages including the short response time and the molecularly resolved nature of resulting information. The latter opens the way to integrate the mass spectrometric profiles into the molecular cancer diagnostic infrastructure by properly identifying significant peaks and linking them to the expression of proteins (enzymes, receptors, transporters) or mutations in genes encoding them.

The current major roadblock regarding the clinical translation of mass spectrometry-based tissue profiling techniques is mostly associated with the unclear regulatory background for multivariate methods and the lack of multicentre studies reported in literature. Additional considerations may arise from the requirements for maintenance of the instruments and development of suitable data processing workflows. Frequent use will demand regular cleaning and servicing cycles that will need to become rapid, simplified and ideally automated. Data processing workflows will need to provide validated, actionable results, quick enough to guide clinical decision making. The identification of the molecular background of MS information and further spreading of the technologies in the biomedical world will likely solve these problems in the course of the following 5–10 years.

References

[1] J. Landercasper, E. Whitacre, A.C. Degnim, M. Al-Hamadani, Reasons for re-excision after lumpectomy for breast cancer: insight from the American Society of Breast Surgeons Mastery(SM) database, Ann. Surg. Oncol. 21 (2014) 3185–3191.

[2] R. Jeevan, D.A. Cromwell, M. Trivella, G. Lawrence, O. Kearins, J. Pereira, et al., Reoperation rates after breast conserving surgery for breast cancer among women in England: retrospective study of hospital episode statistics, BMJ 345 (2012) e4505.

[3] T.P. Olson, J. Harter, A. Munoz, D.M. Mahvi, T. Breslin, Frozen section analysis for intraoperative margin assessment during breast-conserving surgery results in low rates of re-excision and local recurrence, Ann. Surg. Oncol. 14 (2007) 2953–2960.

[4] F. D'Halluin, P. Tas, S. Rouquette, C. Bendavid, F. Foucher, H. Meshba, et al., Intra-operative touch preparation cytology following lumpectomy for breast cancer: a series of 400 procedures, Breast 18 (2009) 248–253.

[5] B. Mesurolle, M. El-Khoury, D. Hori, J.P. Phancao, S. Kary, E. Kao, et al., Sonography of postexcision specimens of nonpalpable breast lesions: value, limitations, and description of a method, AJR Am. J. Roentgenol. 186 (2006) 1014–1024.

[6] K. Mosterd, G.A. Krekels, F.H. Nieman, J.U. Ostertag, B.A. Essers, C.D. Dirksen, et al., Surgical excision versus Mohs' micrographic surgery for primary and recurrent basal-cell carcinoma of the face: a prospective randomised controlled trial with 5-years' follow-up, Lancet Oncol. 9 (2008) 1149–1156.

[7] J.A. Zitelli, R.L. Moy, E. Abell, The reliability of frozen sections in the evaluation of surgical margins for melanoma, J. Am. Acad. Dermatol. 24 (1991) 102–106.

[8] R.J. Barlow, C.R. White, N.A. Swanson, Mohs' micrographic surgery using frozen sections alone may be unsuitable for detecting single atypical melanocytes at the margins of melanoma in situ, Br. J. Dermatol. 146 (2002) 290–294.

[9] G. Ciccarelli, M.R. Di Virgilio, S. Menna, L. Garretti, A. Ala, R. Giani, et al., Radiography of the surgical specimen in early stage breast lesions: diagnostic reliability in the analysis of the resection margins, Radiol. Med. (Torino) 112 (2007) 366–376.

[10] R. Tang, S.B. Coopey, J.M. Buckley, O.P. Aftreth, L.J. Fernandez, E.F. Brachtel, et al., A pilot study evaluating shaved cavity margins with micro-computed tomography: a novel method for predicting lumpectomy margin status intraoperatively, Breast J. 19 (2013) 485–489.

[11] T.E. Doyle, R.E. Factor, C.L. Ellefson, K.M. Sorensen, B.J. Ambrose, J.B. Goodrich, et al., High-frequency ultrasound for intraoperative margin assessments in breast conservation surgery: a feasibility study, BMC Cancer 11 (2011) 444.

[12] D.J. Evers, R. Nachabe, M.J. Vranken Peeters, J.A. van der Hage, H.S. Oldenburg, E.J. Rutgers, et al., Diffuse reflectance spectroscopy: towards clinical application in breast cancer, Breast Cancer Res. Treat. 137 (2013) 155–165.

[13] L.G. Wilke, J.Q. Brown, T.M. Bydlon, S.A. Kennedy, L.M. Richards, M.K. Junker, et al., Rapid noninvasive optical imaging of tissue composition in breast tumor margins, Am. J. Surg. 198 (2009) 566–574.

[14] A.S. Haka, Z. Volynskaya, J.A. Gardecki, J. Nazemi, R. Shenk, N. Wang, et al., Diagnosing breast cancer using Raman spectroscopy: prospective analysis, J. Biomed. Opt. 14 (2009) 054023.

[15] M. Jermyn, K. Mok, J. Mercier, J. Desroches, J. Pichette, K. Saint-Arnaud, et al., Intraoperative brain cancer detection with Raman spectroscopy in humans, Sci. Transl. Med. 7 (2015) 274ra219.

[16] F.T. Nguyen, A.M. Zysk, E.J. Chaney, J.G. Kotynek, U.J. Oliphant, F.J. Bellafiore, et al., Intraoperative evaluation of breast tumor margins with optical coherence tomography, Cancer Res. 69 (2009) 8790–8796.

[17] N. Sanai, J. Eschbacher, G. Hattendorf, S.W. Coons, M.C. Preul, K.A. Smith, et al., Intraoperative confocal microscopy for brain tumors: a feasibility analysis in humans, Neurosurgery 68 (2011) 282–290 discussion 290.

[18] T.C. Chang, J.J. Liu, S.T. Hsiao, Y. Pan, K.E. Mach, J.T. Leppert, et al., Interobserver agreement of confocal laser endomicroscopy for bladder cancer, J. Endourol. 27 (2013) 598–603.

[19] I. Pappo, R. Spector, A. Schindel, S. Morgenstern, J. Sandbank, L.T. Leider, et al., Diagnostic performance of a novel device for real-time margin assessment in lumpectomy specimens, J. Surg. Res. 160 (2010) 277–281.

[20] F. Schnabel, S.K. Boolbol, M. Gittleman, T. Karni, L. Tafra, S. Feldman, et al., A randomized prospective study of lumpectomy margin assessment with use of MarginProbe in patients with nonpalpable breast malignancies, Ann. Surg. Oncol. 21 (2014) 1589–1595.

[21] R. Castaing, G. Slodzian, Microanalyse par emission ionique secondaire, J. Microscopie. 1 (1962) 395–410.

[22] H. Liebl, Ion microprobe mass analyzer, J. Appl. Phys. 38 (1967) 5277–5283.

[23] S.G. Boxer, M.L. Kraft, P.K. Weber, Advances in imaging secondary ion mass spectrometry for biological samples, Annu. Rev. Biophys. 38 (2009) 53–74.

[24] E. Armitage, H. Kotze, N. Lockyer, Imaging of metabolites using secondary ion mass spectrometry, Metabolomics 9 (2013) 102–109.

[25] A.F. Altelaar, I. Klinkert, K. Jalink, R.P. de Lange, R.A. Adan, R.M. Heeren, et al., Gold-enhanced biomolecular surface imaging of cells and tissue by SIMS and MALDI mass spectrometry, Anal. Chem. 78 (2006) 734–742.

[26] M.L. Pacholski, D.M. Cannon Jr., A.G. Ewing, N. Winograd, Static time-of-flight secondary ion mass spectrometry imaging of freeze-fractured, frozen-hydrated biological membranes, Rapid Commun. Mass Spectrom. 12 (1998) 1232–1235.

[27] P. Sjovall, J. Lausmaa, B. Johansson, Mass spectrometric imaging of lipids in brain tissue, Anal. Chem. 76 (2004) 4271–4278.

[28] S.R. Oppenheimer, D. Mi, M.E. Sanders, R.M. Caprioli, Molecular analysis of tumor margins by MALDI mass spectrometry in renal carcinoma, J. Proteome Res. 9 (2010) 2182–2190.

[29] R.L. Caldwell, A. Gonzalez, S.R. Oppenheimer, H.S. Schwartz, R.M. Caprioli, Molecular assessment of the tumor protein microenvironment using imaging mass spectrometry, Cancer Genomics Proteomics 3 (2006) 279–287.

[30] M.H. Zhang, X.H. Xu, Y. Wang, Q.X. Linq, Y.T. Bi, X.J. Miao, et al., A prognostic biomarker for gastric cancer with lymph node metastases, Anat. Rec. (Hoboken) 296 (2013) 590–594.

[31] G.K. Lee, H.S. Lee, Y.S. Park, J.H. Lee, S.C. Lee, J.H. Lee, et al., Lipid MALDI profile classifies non-small cell lung cancers according to the histologic type, Lung Cancer 76 (2012) 197–203.

[32] K. Schwamborn, R.C. Krieg, J. Grosse, N. Reulen, R. Weiskirchen, R. Knuechel, et al., Serum proteomic profiling in patients with bladder cancer, Eur. Urol. 56 (2009) 989–996.

[33] K. Chughtai, L. Jiang, T.R. Greenwood, K. Glunde, R.M. Heeren, Mass spectrometry images acylcarnitines, phosphatidylcholines, and sphingomyelin in MDA-MB-231 breast tumor models, J. Lipid Res. 54 (2013) 333–344.

[34] R. Longuespee, C. Boyon, C. Castellier, A. Jacquet, A. Desmons, O. Kerdraon, et al., The C-terminal fragment of the immunoproteasome PA28S (Reg alpha) as an early diagnosis and tumor-relapse biomarker: evidence from mass spectrometry profiling, Histochem. Cell Biol. 138 (2012) 141–154.

[35] M. Kazmierczak, M. Luczak, K. Lewandowski, L. Handschuh, A. Czyz, M. Jarmuz, et al., Esterase D and gamma 1 actin level might predict results of induction

therapy in patients with acute myeloid leukemia without and with maturation, Med. Oncol. 30 (2013) 725.
[36] Z. Takats, J.M. Wiseman, B. Gologan, R.G. Cooks, Mass spectrometry sampling under ambient conditions with desorption electrospray ionization, Science 306 (2004) 471–473.
[37] J.M. Wiseman, S.M. Puolitaival, Z. Takáts, R.G. Cooks, R.M. Caprioli, Mass spectrometric profiling of intact biological tissue by using desorption electrospray ionization, Angew. Chem. Int. Ed. Engl. 117 (2005) 7256–7259.
[38] L.S. Eberlin, A.L. Dill, A.B. Costa, D.R. Ifa, L. Cheng, T. Masterson, et al., Cholesterol sulfate imaging in human prostate cancer tissue by desorption electrospray ionization mass spectrometry, Anal. Chem. 82 (2010) 3430–3434.
[39] S. Santagata, L.S. Eberlin, I. Norton, D. Calligaris, D.R. Feldman, J.L. Ide, et al., Intraoperative mass spectrometry mapping of an onco-metabolite to guide brain tumor surgery, Proc. Natl. Acad. Sci. U.S.A. 111 (2014) 11121–11126.
[40] D. Calligaris, D. Caragacianu, X. Liu, I. Norton, C.J. Thompson, A.L. Richardson, et al., Application of desorption electrospray ionization mass spectrometry imaging in breast cancer margin analysis, Proc. Natl. Acad. Sci. U.S.A. 111 (2014) 15184–15189.
[41] L.S. Eberlin, R.J. Tibshirani, J. Zhang, T.A. Longacre, G.J. Berry, D.B. Bingham, et al., Molecular assessment of surgical-resection margins of gastric cancer by mass-spectrometric imaging, Proc. Natl. Acad. Sci. U.S.A. 111 (2014) 2436–2441.
[42] P.S. Ward, J. Patel, D.R. Wise, O. Abdel-Wahab, B.D. Bennett, H.A. Coller, et al., The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting alpha-ketoglutarate to 2-hydroxyglutarate, Cancer Cell 17 (2010) 225–234.
[43] C. Choi, S.K. Ganji, R.J. DeBerardinis, K.J. Hatanpaa, D. Rakheja, Z. Kovacs, et al., 2-hydroxyglutarate detection by magnetic resonance spectroscopy in IDH-mutated patients with gliomas, Nat. Med. 18 (2012) 624–629.
[44] D. Capper, S. Weissert, J. Balss, A. Habel, J. Meyer, D. Jager, et al., Characterization of R132H mutation-specific IDH1 antibody binding in brain tumors, Brain Pathol. 20 (2010) 245–254.
[45] A.K. Jarmusch, V. Pirro, Z. Baird, E.M. Hattab, A.A. Cohen-Gadol, R.G. Cooks, Lipid and metabolite profiles of human brain tumors by desorption electrospray ionization-MS, Proc. Natl. Acad. Sci. U.S.A. 113 (2016) 1486–1491.
[46] L.S. Eberlin, I. Norton, A.L. Dill, A.J. Golby, K.L. Ligon, S. Santagata, et al., Classifying human brain tumors by lipid imaging with mass spectrometry, Cancer Res. 72 (2012) 645–654.
[47] L.S. Eberlin, I. Norton, D. Orringer, I.F. Dunn, X. Liu, J.L. Ide, et al., Ambient mass spectrometry for the intraoperative molecular diagnosis of human brain tumors, Proc. Natl. Acad. Sci. U.S.A. 110 (2013) 1611–1616.
[48] D. Calligaris, I. Norton, D.R. Feldman, J.L. Ide, I.F. Dunn, L.S. Eberlin, et al., Mass spectrometry imaging as a tool for surgical decision-making, J. Mass Spectrom. 48 (2013) 1178–1187.
[49] F. Bregeon, G. Brioude, F. De Dominicis, T. Atieh, X.B.D. Journo, C. Flaudrops, et al., MALDI-ToF mass spectrometry for the rapid diagnosis of cancerous lung nodules, PLoS ONE 9 (2014) e97511.
[50] Y. Ide, M. Waki, T. Hayasaka, T. Nishio, Y. Morita, H. Tanaka, et al., Human breast cancer tissues contain abundant phosphatidylcholine(36ratio1) with high stearoyl-CoA desaturase-1 expression, PLoS One 8 (2013) e61204.
[51] R. Mirnezami, K. Spagou, P.A. Vorkas, M.R. Lewis, J. Kinross, E. Want, et al., Chemical mapping of the colorectal cancer microenvironment via MALDI imaging mass spectrometry (MALDI-MSI) reveals novel cancer-associated field effects, Mol. Oncol. 8 (2014) 39–49.
[52] D. Calligaris, D.R. Feldman, I. Norton, O. Olubiyi, A.N. Changelian, R. Machaidze, et al., MALDI mass spectrometry imaging analysis of pituitary adenomas for near-real-time tumor delineation, Proc. Natl. Acad. Sci. U.S.A. 112 (2015) 9978–9983.
[53] C.H. Chen, Z. Lin, S. Garimella, L. Zheng, R. Shi, R.G. Cooks, et al., Development of a mass spectrometry sampling probe for chemical analysis in surgical and endoscopic procedures, Anal. Chem. 85 (2013) 11843–11850.
[54] N.Y. Agar, A.J. Golby, K.L. Ligon, I. Norton, V. Mohan, J.M. Wiseman, et al., Development of stereotactic mass spectrometry for brain tumor surgery, Neurosurgery 68 (2011) 280–289 discussion 290.
[55] S. Guenther, L.J. Muirhead, A.V. Speller, O. Golf, N. Strittmatter, R. Ramakrishnan, et al., Spatially resolved metabolic phenotyping of breast cancer by desorption electrospray ionization mass spectrometry, Cancer Res. 75 (2015) 1828–1837.
[56] K.C. Sachfer, T. Szaniszlo, S. Gunther, J. Balog, J. Denes, M. Keseru, et al., In situ, real-time identification of biological tissues by ultraviolet and infrared laser desorption ionization mass spectrometry, Anal. Chem. 83 (2011) 1632–1640.
[57] K.C. Schafer, J. Balog, T. Szaniszlo, D. Szalay, G. Mezey, J. Denes, et al., Real time analysis of brain tissue by direct combination of ultrasonic surgical aspiration and sonic spray mass spectrometry, Anal. Chem. 83 (2011) 7729–7735.
[58] J. Balog, L. Sasi-Szabo, J. Kinross, M.R. Lewis, L.J. Muirhead, K. Veselkov, et al., Intraoperative tissue identification using rapid evaporative ionization mass spectrometry, Sci. Transl. Med. 5 (2013) 194ra193.
[59] H. Wang, N.E. Manicke, Q. Yang, L. Zheng, R. Shi, R.G. Cooks, et al., Direct analysis of biological tissue by paper spray mass spectrometry, Anal. Chem. 83 (2011) 1197–1201.
[60] K. Yoshimura, L.C. Chen, M.K. Mandal, T. Nakazawa, Z. Yu, T. Uchiyama, et al., Analysis of renal cell carcinoma as a first step for developing mass spectrometry-based diagnostics, J. Am. Soc. Mass Spectrom. 23 (2012) 1741–1749.
[61] M.K. Mandal, S. Saha, K. Yoshimura, Y. Shida, S. Takeda, H. Nonami, et al., Biomolecular analysis and cancer diagnostics by negative mode probe electrospray ionization, Analyst 138 (2013) 1682–1688.
[62] G.A. Gomez-Rios, J. Pawliszyn, Development of coated blade spray ionization mass spectrometry for the quantitation of target analytes present in complex matrices, Angew. Chem. Int. Ed. Engl. 53 (2014) 14503–14507.
[63] J. Deng, Y. Yang, M. Xu, X. Wang, L. Lin, Z.P. Yao, et al., Surface-coated probe nanoelectrospray ionization mass spectrometry for analysis of target compounds in individual small organisms, Anal. Chem. 87 (2015) 9923–9930.
[64] G.A. Gomez-Rios, N. Reyes-Garces, B. Bojko, J. Pawliszyn, Biocompatible solid-phase microextraction nanoelectrospray ionization: an unexploited tool in bioanalysis, Anal. Chem. 88 (2016) 1259–1265.
[65] R.W. Ryan, T. Wolf, R.F. Spetzler, S.W. Coons, Y. Fink, M.C. Preul, Application of a flexible CO(2) laser fiber for neurosurgery: laser-tissue interactions, J. Neurosurg. 112 (2010) 434–443.
[66] J.T. Au, A. Mittra, J. Wong, S. Carpenter, J. Carson, D. Haddad, et al., Flexible CO2 laser and submucosal gel injection for safe endoluminal resection in the intestines, Surg. Endosc. 26 (2012) 47–52.
[67] K. Hiraoka, K. Nishidate, K. Mori, D. Asakawa, S. Suzuki, Development of probe electrospray using a solid needle, Rapid Commun. Mass Spectrom. 21 (2007) 3139–3144.
[68] X. Gong, Y. Zhao, S. Cai, S. Fu, C. Yang, S. Zhang, et al., Single cell analysis with probe ESI-mass spectrometry: detection of metabolites at cellular and subcellular levels, Anal. Chem. 86 (2014) 3809–3816.
[69] B. Bojko, K. Gorynski, G.A. Gomez-Rios, J.M. Knaak, T. Machuca, E. Cudjoe, et al., Low invasive in vivo tissue sampling for monitoring biomarkers and drugs during surgery, Lab. Invest. 94 (2014) 586–594.
[70] É.A. Souza-Silva, N. Reyes-Garcés, G.A. Gómez-Ríos, E. Boyacı, B. Bojko, J. Pawliszyn, A critical review of the state of the art of solid-phase microextraction of complex matrices III. Bioanalytical and clinical applications, Trends Analyt. Chem. 71 (2015) 249–264.