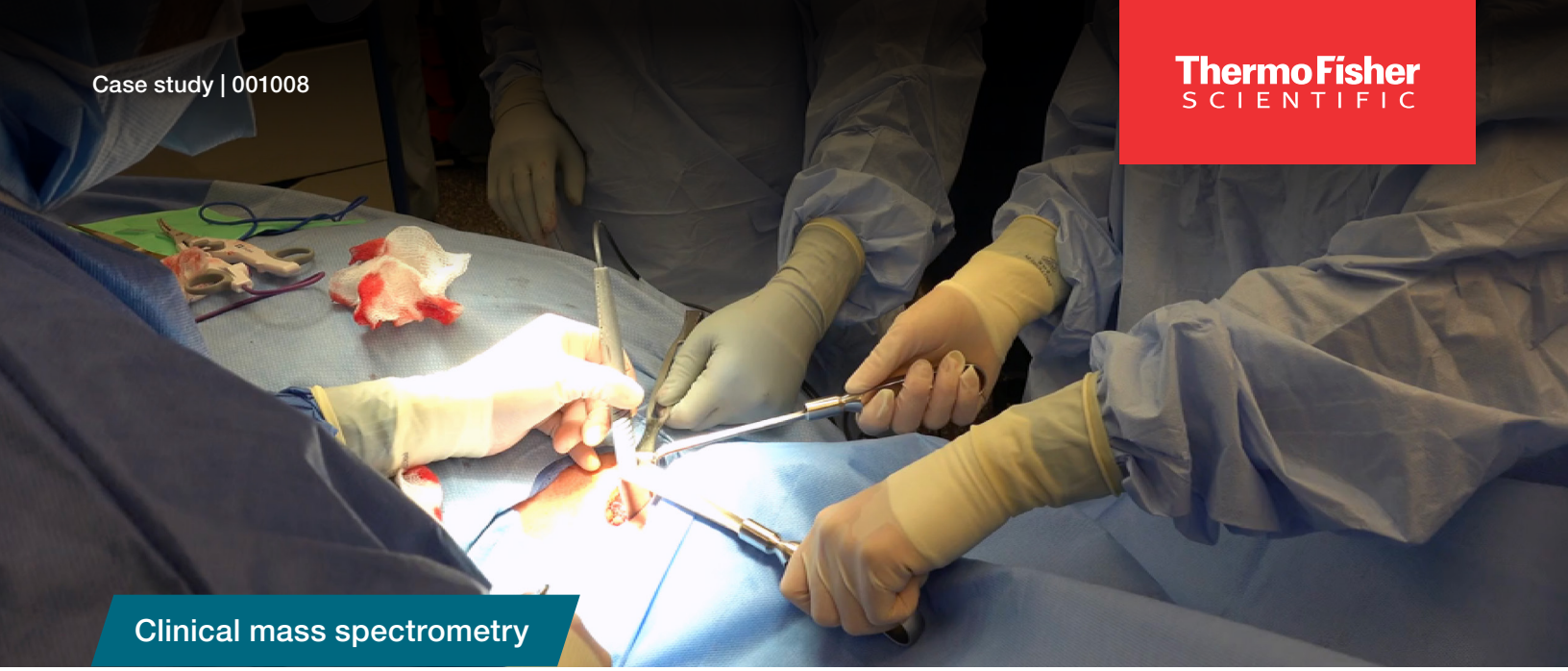

Clinical mass spectrometry

Photo courtesy of Eberlin Laboratory, Baylor College of Medicine.

The MasSpec Pen in the hands of surgeons

Real-time sensitive, specific, and accurate identification of cancerous tissue

Key point summary

The MasSpec Pen System™:

- Is accurate and reproducible
- Provides a user-friendly mass spectrometry interface for surgeons
- Moves intraoperative tissue analysis closer to patient care
- Allows non-destructive analysis of tissues *in vivo*
- Could advance intraoperative decision making for surgeons treating cancer patients
- Decreases turnaround time during surgical tumor margin evaluation
- May improve patient outcomes

What is the MasSpec Pen?

Background

Mass spectrometers versus microscopes: the use of spectra versus stains in the management of surgical histopathology could give surgeons and pathologists an advantage of real-time identification of cancerous versus normal tissue while reducing surgical time and improving patient care. Intraoperative histopathology (*ex vivo* frozen tissue section analysis) has been the standard of care for disease-based tissue analysis for over 100 years and is typically used for tumor identification from excised tissue during surgical resection. Intraoperative histopathology analysis of normal and diseased tissue involves removing a piece of tissue from an individual and then freezing, sectioning, and staining the tissue with either hematoxylin and eosin (H&E) or by immunohistochemistry (IHC). This process is then followed by a pathologist's review of the tissue section under a microscope, with results reported back to the surgeon in the operating room. Time to results during surgery is about 15–45 minutes. Therefore, it is only practical for a few samples to be evaluated during a surgery. In addition, artifacts due to the freezing and tissue processing steps, as well as complex histopathology such as in pancreatic cancer tissue, can complicate analysis.

> "We really want to put mass spectrometry technology into the hands of surgeons and medical professionals."
>
> -Dr. Livia Eberlin

Photo courtesy of Eberlin Laboratory, Baylor College of Medicine.

Direct mass spectrometry—the future of point-of-care diagnostics

The MasSpec Pen provides a new mass-spectrometry based approach to address this problem of characterization of cancerous tissue during resection in the operating room. For surgeons performing cancer tissue resection, the goal is to remove all cancerous tissue while minimizing the removal of normal tissue. In up to 15–30% of surgically resected tissues that are visually determined to have clear margins by the surgeon in the operating room, a histopathology review by a pathologist finds the tissue does not have clear margins. If this process of tissue analysis during surgery could be interfaced with surgical decision making and performed in real-time, surgeons could receive immediate feedback as to whether or not the tissue margins are tumor-free without long wait times. A mass spectrometer could provide this type of immediate feedback, but traditional instruments are big, complex to operate, and not designed to be used directly on *in vivo* patient tissue in an operating room. However, the development of ambient ionization mass spectrometry techniques is driving the adoption of mass spectrometry into the clinical and surgical space. Ambient ionization mass spectrometry techniques include sample analysis and ionization methods that require almost no sample preparation and provide almost immediate sample acquisition in the open-air. The MasSpec Pen technology employs similar principles as it can be used to directly analyze unmodified tissue samples *in vivo* and *ex vivo*.[1]

Dr. Livia Eberlin, who developed the MasSpec Pen with her research team and collaborators, explains, "What was really lacking and that we aimed to address with the MasSpec Pen technology was a simple device between the mass spectrometer and the surgeon that was easy to use, biocompatible, and was something that the surgeons could routinely employ in surgery without having training in mass spectrometry. In under one minute you can get an answer to a clinical problem that would be really challenging with other technologies. What is also incredible is that the MasSpec Pen allows surgeons to perform non-destructive molecular analysis and identification of tissues *in vivo* to identify tissues even before resection, a capability currently unavailable in surgical practice."

About the MasSpec Pen

Dr. Eberlin has had a longstanding research collaboration with Thermo Fisher Scientific, initiated at the early stages of the MasSpec Pen development in 2016. As longtime users of Orbitrap™ mass spectrometers, Eberlin and her team have integrated the MasSpec Pen to many different models of Orbitrap instrumentation, and have had recent success in the clinical translation of the MasSpec Pen technology with the Thermo Scientific™ Orbitrap Exploris™ systems. Current clinical studies at Baylor College of Medicine and MD Anderson Cancer Center are being performed with the Orbitrap Exploris 120 and the Orbitrap Exploris 240 mass spectrometers coupled to the MasSpec Pen system, respectively. This allows surgeons to acquire high-resolution mass spectrometry data with the MasSpec Pen device during surgical procedures.

The MasSpec Pen is a hand-held device that acts as an intuitive interface between the surgeon and a mass spectrometer to provide rapid information on tissue identification and cancer diagnostics. The hand-held device can be directly connected to the MasSpec Pen interface that is integrated to a high-resolution mass spectrometer, such as the Orbitrap Exploris 120 mass spectrometer (Figure 1). The device is disposable and autoclavable for repeat use. It is biocompatible and its use is non-destructive to tissue. Analysis with the MasSpec Pen is automated and provides information in approximately 10–20 seconds.

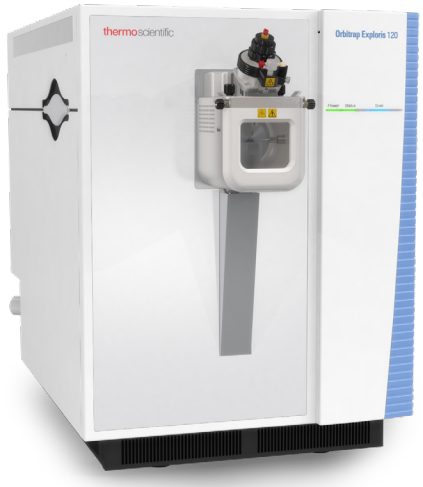

Figure 1. The Orbitrap Exploris series mass spectrometers provide high resolution and mass accuracy as well as consistent reproducibility and operational simplicity with a compact footprint. This enables integration into a clinical setting that otherwise would not be able to house a mass spectrometer, such as an operating room, making it the ideal mass spectrometer to pair with the MasSpec Pen.

The device typically uses 20–50 µL of water in a liquid-solid extraction process to extract biomolecules from tissue and then directs the extract to the mass spectrometer via tubing.

The mass spectrometer is most commonly operated in the negative ion mode because many of the metabolites and lipids detected in the mass spectral profiles related to cancer metabolism are ionized in that polarity. The analysis typically has a low protein profile, which simplifies analysis.

The pen tip of the MasSpec Pen device consists of three separate channels—one each for incoming water, gas (N_2, CO_2, or air), and the outgoing droplet—that merge into one channel, a reservoir. Molecular extraction takes place in the reservoir, located at the tip of the MasSpec Pen, where a water droplet is retained and used to extract biomolecules directly from the tissue surface. The gas channel is used to compensate for the vacuum created by the mass spectrometer when the outgoing droplet and extracted biomolecules are transported to the MasSpec Pen interface within the Orbitrap mass spectrometer (Figure 2).

Dr. Livia Eberlin

Dr. Livia Eberlin's passion is developing and translating mass spectrometry technology for clinical use to improve patient care. She is an Associate Professor in the Department of Surgery at Baylor College of Medicine. Dr. Eberlin's father is a chemist trained in mass spectrometry and her mother was a biochemist. Growing up, she was fascinated with molecules and how they made up our biological systems. She realized that she wanted to take advantage of the power of the mass spectrometer to make an impact in clinical care for cancer patients.

When asked, "Why use mass spectrometry?" Dr. Eberlin responds, "In what I do, which is metabolic analysis, there are incredible differences between cancer and normal cells that might not be picked up by histological analysis, but mass spectrometry can pick these up in real-time." The MasSpec Pen was developed to address these challenges associated with traditional intraoperative histopathology tissue analysis by providing transformative capability of molecular-based tissue assessment *in vivo*, in the operating room. Currently the MasSpec Pen is used as a research device at Baylor College of Medicine, MD Anderson Cancer Center, and Johns Hopkins University.

Photos courtesy of Eberlin Laboratory, Baylor College of Medicine.

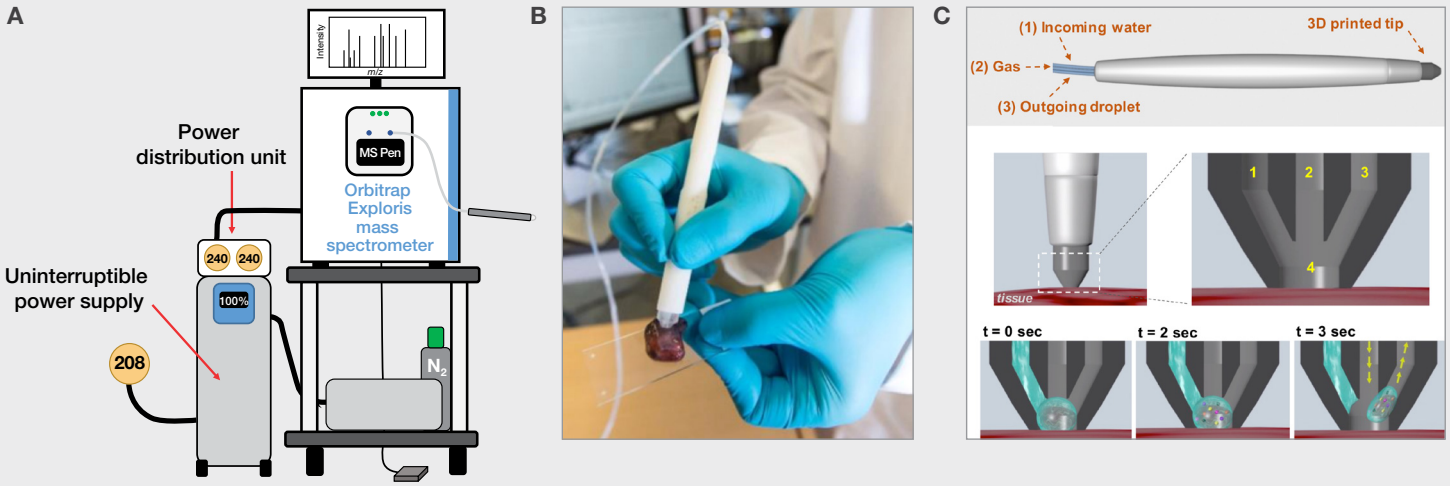

Figure 2. (A) The MasSpec Pen device is directly connected to the MasSpec Pen interface that is integrated to the Orbitrap mass spectrometer. (B) Analysis with the MasSpec Pen device is triggered by a foot pedal, which directly communicates with the control systems within the MasSpec Pen interface. (C) Cutaway view showing channels for water, air, and sample intake directed to the interface within the Orbitrap mass spectrometer. *Diagrams courtesy of Eberlin Laboratory, Baylor College of Medicine.*

This tissue sampling process is easily controlled by a foot pedal on the floor near the user.[2] The Orbitrap Exploris 120 mass spectrometer is used to obtain high mass accuracy spectra, which is vital when analyzing metabolites, lipids, and other biomolecules. The high resolution and mass accuracy of the Orbitrap system is critical in the analysis of complex samples like tissues, which facilitates the interpretation of the spectral data. Once the mass spectra is acquired by the mass spectrometer, the Lasso logistic regression algorithm is used to interpret the spectral data and provide a prediction of disease-state or tissue identification.

There are numerous medical and surgical applications for the MasSpec Pen, including intraoperative differentiation of cancer tissue from normal tissue, surgical identification of metastatic lesions *in vivo*, identifying tumor-free tissue (clear margins) in the resection of cancerous tissue, and identification of visually indistinguishable tissue for surgical removal such as in the case of distinguishing parathyroid from thyroid tissue. Some of the specific disease applications for which the MasSpec Pen has been used include ovarian cancer, invasive ductal carcinoma, pancreatic ductal adenocarcinoma, endocrine diseases, and brain cancer.

The MasSpec Pen technology is currently being commercialized by MS Pen Technologies Inc., a startup company in Texas Medical Center in Houston.

Rapid, robust, and reproducible characterization of banked *ex vivo* cancer tissues

Building a model to compare normal tissue to cancer tissue using banked tissue samples

In a study performed in ovarian cancer, identification and differentiation between cancer and normal tissue were initially performed on banked tissue samples to identify mass spectral patterns that were characteristic of tissue type and develop statistical models. Then, a banked tissue sample containing regions of high-grade serous carcinoma (HGSC) adjacent to normal ovarian tissue was used to evaluate the potential ability of the MasSpec Pen to identify margins between normal and cancerous tissues.

In the banked tissue sample, regions 1 and 2 were classified as histologically normal. Regions 4 and 5 were histologically classified as HGSC. Region 3 was within the tumor margin and contained approximately 50% normal ovarian stroma tissue and 50% tumor tissue. Using the MasSpec Pen, regions 1 and 2 were correctly identified as normal tissue and regions 3, 4, and 5 were correctly identified as tumor-containing tissue. Results from this study using banked ovarian tissue samples support the potential for the MasSpec Pen to intraoperatively identify tissue margins in real-time.[2]

Using the MasSpec Pen to quickly differentiate ovarian cancer subtypes (HGSC vs. LGSC) and determine treatment options

Rapid diagnosis and subtyping of ovarian cancer is essential to defining treatment options. From banked or previously collected ovarian tissue, a 3-class statistical model (normal ovarian tissue, low-grade serous ovarian carcinoma [LGSC], and HGSC) was developed to differentiate the subtypes of ovarian cancer. Select metabolites used to generate the statistical models included ascorbate and taurine for normal tissue; lactate, glutathione and glycerophosphoinositol (PI) 18:0_20:4 were used for HGSC; and glycerophosphoethanolamine (PE) P-18:0_20:4 for LGSC. Gluconate and glutamate were selected to identify both HGSC and LGSC (Figure 3). All normal ovarian tissue and HGSC tissue were correctly classified (100% accuracy). Low-grade serous ovarian carcinoma tissue was classified correctly with an accuracy of 71.4%. The lower accuracy of LGSC may be due to the limited number of LGSC tissue samples available in the study (n = 7) to develop the statistical prediction model. Overall, the 3-class model had 97.7% accuracy for cancer diagnosis.[3]

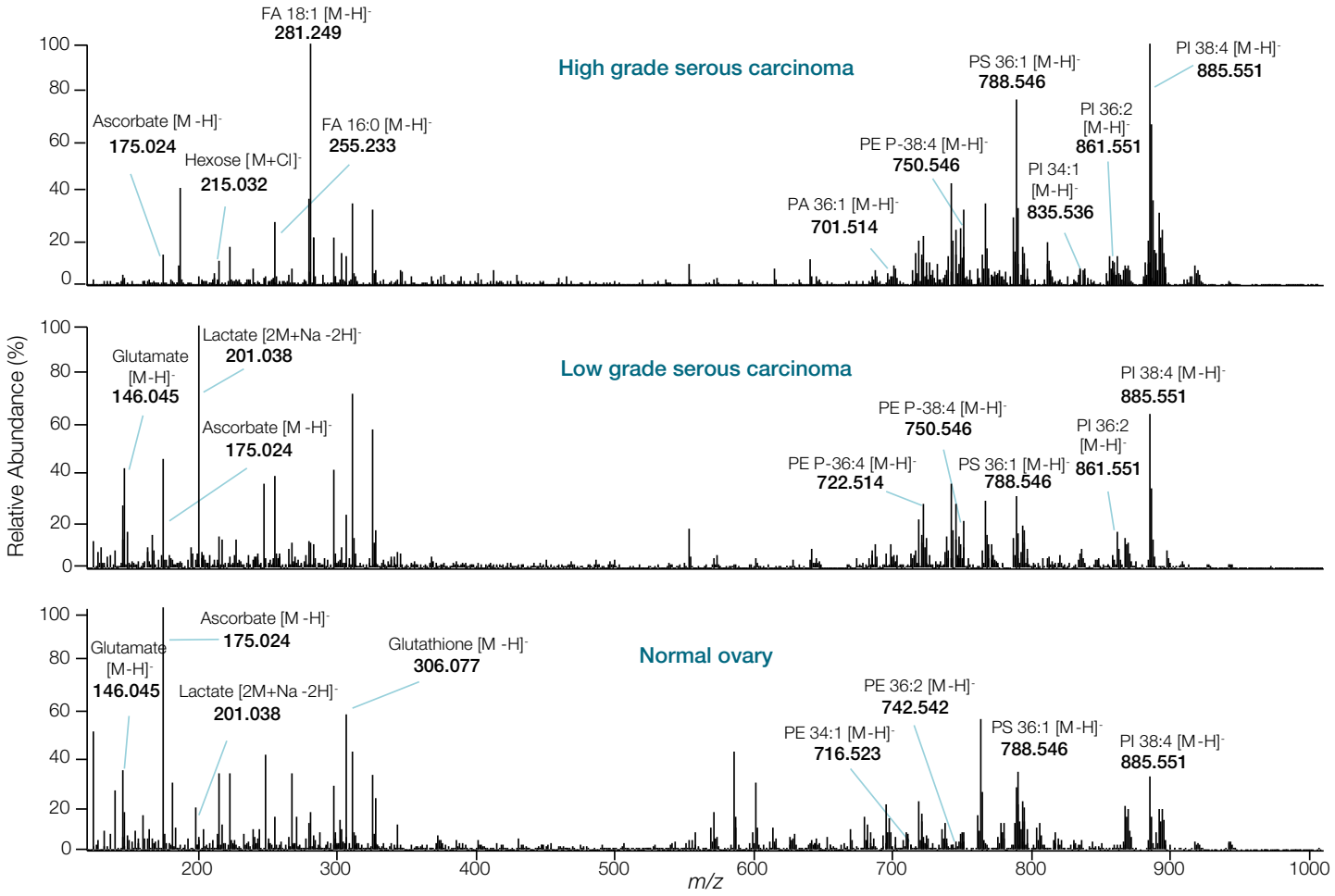

Figure 3. MasSpec Pen acquired spectra from high grade serous ovarian cancer, low grade serous ovarian cancer, and normal ovary.
Spectra courtesy of Eberlin Laboratory, Baylor College of Medicine.

Intraoperative identification of cancerous tissue and surgical margin evaluation

Initial clinical pilot tests have surgeons using the MasSpec Pen in the operating room

The MasSpec Pen was used to diagnose pancreatic ductal adenocarcinoma intraoperatively based on metabolite and lipid profiles generated from the MasSpec Pen. A total of 157 banked pancreatic ductal adenocarcinoma, pancreatic, and bile duct tissues were analyzed with the MasSpec Pen. Lasso logistic regression was used to discriminate pancreatic cancer from normal pancreatic tissue using a few select metabolites that were characteristic of pancreatic cancer tissue. The MasSpec Pen could distinguish normal pancreatic tissue from cancerous tissue with an overall agreement with pathology of 91.5%, sensitivity of 95.5%, and specificity of 89.7%. Bile duct tissue could be distinguished from pancreatic cancer with an overall agreement with pathology of 95%, sensitivity of 92%, and specificity of 100%.[4]

Surgical margin evaluation in pancreatic cancer with Whipple procedure

In this study, a patient with pancreatic ductal adenocarcinoma had undergone a Whipple procedure, which removes the head of the pancreas, duodenum, common bile duct, and gallbladder and reconnects the remaining organs. Using the MasSpec Pen, the tissue margins of the pancreas neck and bile duct body were analyzed *in vivo* intraoperatively by a surgeon (without specialized mass spectrometry training) and sampling occurred at the surgeon's discretion. The results from the MasSpec Pen analysis correlated with the postoperative pathology reports finding no evidence of pancreatic tumor left in the remaining tissue in either of the tissue margins sampled by the surgeon. Overall, 64 analyses from 18 pancreatic surgeries were evaluated in this study with a 93.8% agreement between the MasSpec Pen analysis in the operating room by a surgeon and the postoperative pathology report. When a portion of the intraoperative frozen tissue section data acquired from the operating room was added into the statistical classifiers for the prediction model, 100% agreement was obtained (Figure 4).

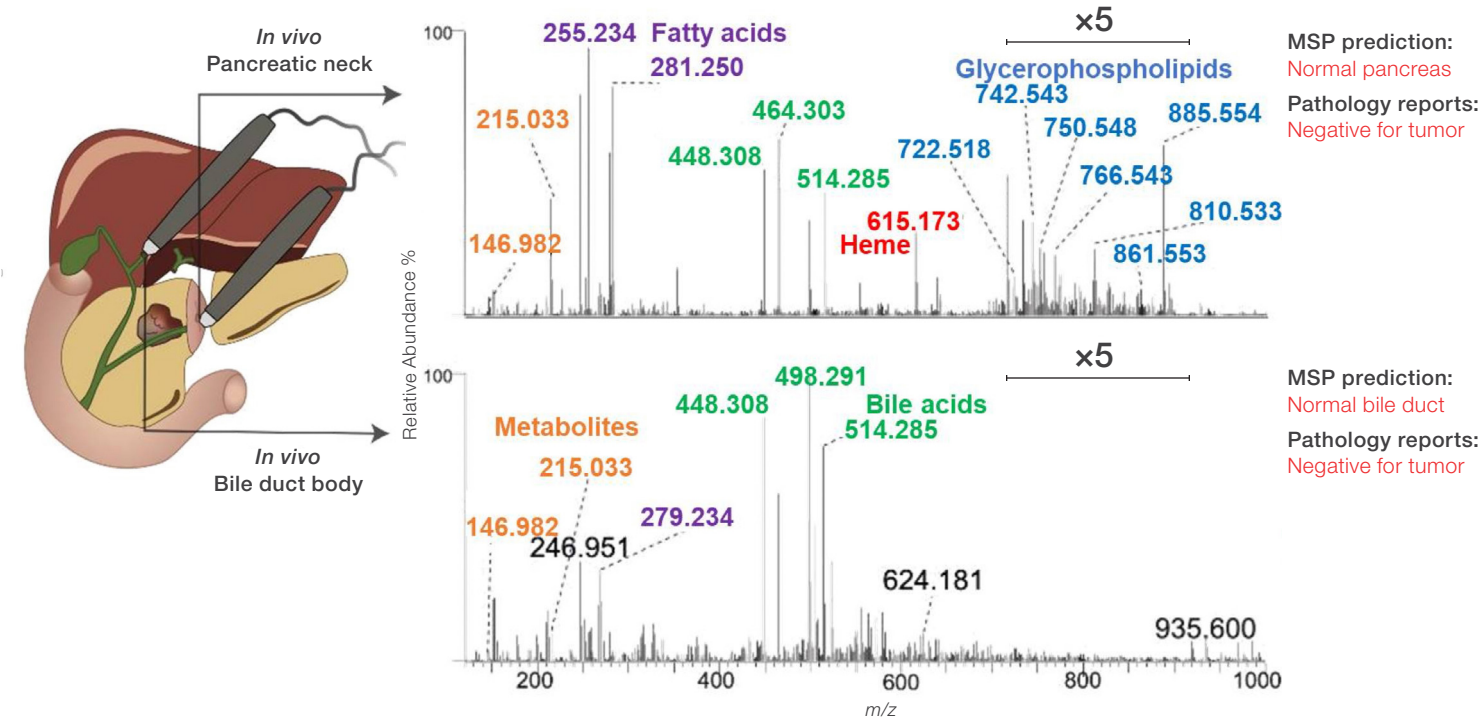

Figure 4. Mass spectra obtained by surgeons using the MasSpec Pen of intraoperative pancreatic neck and bile duct body during a Whipple procedure to assess surgical margins from a patient with pancreatic ductal adenocarcinoma. *Spectra courtesy of Eberlin Laboratory, Baylor College of Medicine.*

Precision medicine translated into the operating room and assessed by surgeons

Pilot clinical study: Use of the MasSpec Pen by an affiliate hospital of Baylor College of Medicine

When envisioning the MasSpec Pen in use in the operating room, Dr. Eberlin explains, "When we developed the MasSpec Pen, the idea was to make it into a very user-friendly device that would allow surgeons, pathologists, and clinicians to take advantage of the power of mass spectrometry technology without requiring a PhD in analytical chemistry." A pilot clinical study took place at an affiliate hospital of Baylor College of Medicine (Houston, TX) to demonstrate that surgeons can independently operate the mass spectrometry system without extensive training and acquire clinically relevant data. While the research team was available for consultation in the operating room, the surgeons were the ones independently operating the MasSpec Pen device *in vivo* during the surgeries. The MasSpec Pen was used during 100 surgical procedures to collect 715 analyses by seven different surgeons and surgical teams. Samples were from *in vivo* and recently excised *ex vivo* normal and diseased thyroid, parathyroid, lymph node, breast, pancreatic, and bile duct tissue samples. The MasSpec Pen was used to acquire mass spectra from the tissues by the surgeons. Small metabolites, lipids, and other biomolecules were identified from these tissue samples and detected in similar relative abundances to the previously characterized data from banked tissues obtained from the research laboratory. No tissue damage was observed upon surgeon or pathologist (gross and microscopic) review. From the patients evaluated, there were no device-related complications reported. "The surgical teams from Baylor indicated the MasSpec Pen analysis procedure was efficient, well-tolerated, and no device specific intraoperative or post-operative complications to patients were reported."[5]

Pilot clinical study: Breast cancer mastectomy

In this pilot clinical study, surgeons independently obtained mass spectrometry data via the MasSpec Pen on a breast cancer patient undergoing a mastectomy. During surgery, the surgeon visually identified a tissue region as potentially cancerous within the breast tissue. The surgeon also analyzed this region *in vivo* with the MasSpec Pen. The data from the MasSpec Pen predicted the tissue identified by the surgeon as potentially cancerous to be of normal breast. A postoperative pathology report revealed that this patient had been treated with neoadjuvant chemotherapy and had a complete response to therapy prior to this surgery. Therefore, there was agreement with the postoperative pathology report and the MasSpec Pen analysis; however, visual identification by the surgeon was discordant (Figure 5).

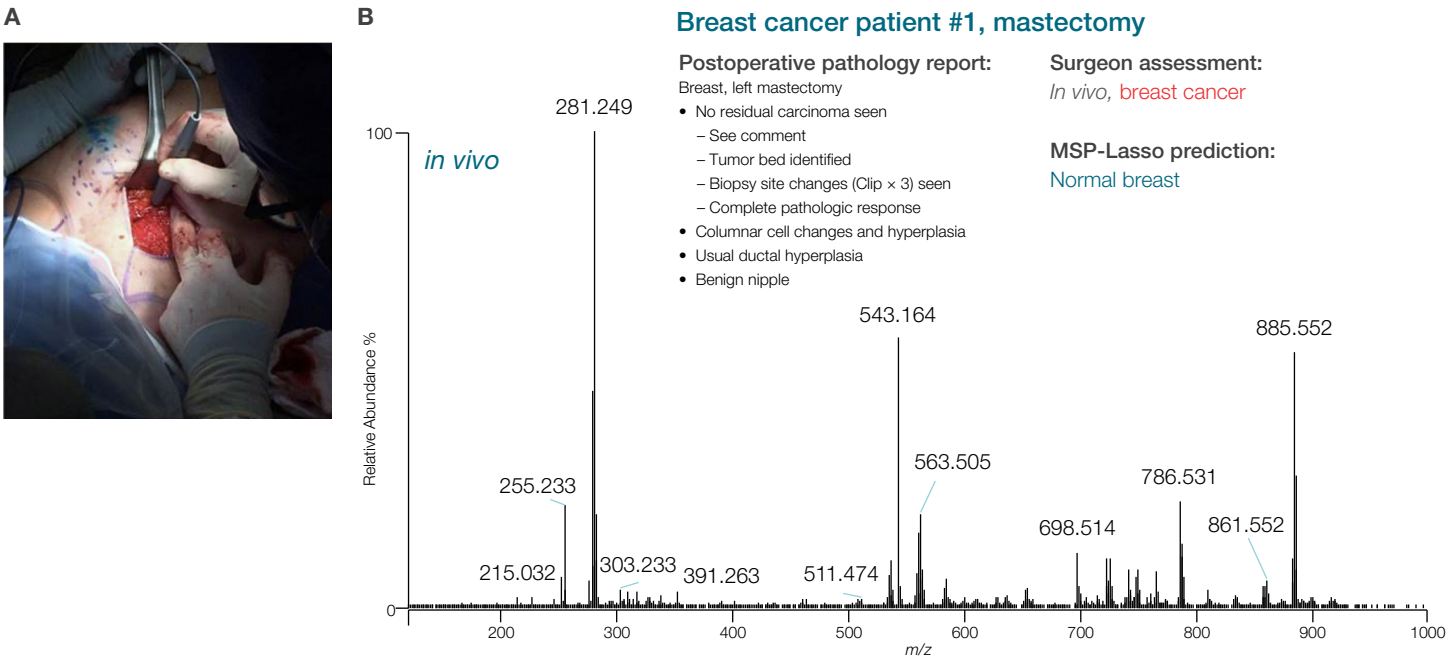

Figure 5. (A) Intraoperative use of the MasSpec Pen during a breast cancer lumpectomy; (B) Mass spectrum of surgeon assessed breast cancer tissue acquired with the MasSpec Pen. *Spectra courtesy of Eberlin Laboratory, Baylor College of Medicine.*

Livia Eberlin with the MasSpec Pen team. Front row: Manoj Kumar, Andreia Porcari, Monica Lin, Livia Eberlin, Anna Krieger, Mike Keating; Middle row: Alena Bensusan, Meredith Spradlin, Rachel DeHoog; Back row: Andy Espinoza, Bjoern Burckhardt, Keziah Liebenberg, Charlie Wolfe, Abby Gatmaitan, Mary King, Trevor Godfrey. *Photos courtesy of Eberlin Laboratory, Baylor College of Medicine.*

Conclusions

- The MasSpec Pen brings intraoperative tissue analysis closer to patient care.
- The MasSpec Pen is easy to use. Surgeons or their surgical team members do not require extensive training to operate the device.
- The MasSpec Pen can be used at the point of care to decrease turnaround time of histopathology review during intraoperative procedures.
- The MasSpec Pen platform has the potential to improve and expedite margin evaluation during cancer surgery in real-time.
- The MasSpec Pen has found its utility in the operating room as a tool to guide surgical decision making leading to improved patient outcomes.
- The Orbitrap Exploris mass spectrometer is the ideal companion for the MasSpec Pen due to its high-resolution performance and its small footprint, which makes it more suitable for use in the operating room.

References:

1. Banerjee, S. Empowering Clinical Diagnostics with Mass Spectrometry. ACS Omega. 2020. https://pubs.acs.org/doi/10.1021/acsomega.9b03764
2. Zhang, J.; Rector, J.; Lin, J.Q.; Young, J.H.; Sans, M.; Katta, N.; Giese, N.; Yu, W.; Nagi, C.; Suliburk, J.; Liu, J.; Bensussan, A.; DeHoog, R.J.; Garza, K.Y.; Ludolph, B.; Sorace, A.G.; Syed, A.; Zahedivash, A.; Milner, T.E.; Eberlin, L.S. Nondestructive tissue analysis for *ex vivo* and *in vivo* cancer diagnosis using a handheld mass spectrometry system. Sci. Transl. Med. 2017 Sep 6, 9(406). https://www.science.org/doi/10.1126/scitranslmed.aan3968
3. Sans, M.; Zhang, J.; Lin, J.Q.; Feider, C.L.; Giese, N.; Breen, M.T.; Sebastian, K.; Liu, J.; Sood, A.K.; Eberlin, L.S. Performance of the MasSpec Pen for Rapid Diagnosis of Ovarian Cancer, Clin. Chem. 2019, 65(5), 674–683. https://doi.org/10.1373/clinchem.2018.299289
4. King, M.E.; Zhang, J.; Lin, J.Q.; Garza, K.Y.; DeHoog, R.J.; Feider, C.L.; Bensussan, A.; Sans, M.; Krieger, A.; Badal, S.; Keating, M.F.; Woody, S.; Dhingra, S.; Yu, W.; Pirko, C.; Brahmbhatt, K.A.; Van Buren, G.; Fisher, W.E.; Suliburk, J.; Eberlin, L.S. Rapid Diagnosis and Tumor Margin Assessment During Pancreatic Cancer Surgery with the MasSpec Pen Technology. Proc. Natl. Acad. Sci. USA. 2021 Jul 13, 118(28). https://doi.org/10.1073/pnas.2104411118
5. Zhang, J.; Sans, M.; DeHoog, R.J.; Garza, K.Y.; King, M.E.; Feider, C.L.; Bensussan, A.; Keating, M.F.; Lin, J.Q.; Povilaitis, S.C.; Katta, N.; Milner, T.E.; Yu, W.; Nagi, C.; Dhingra, S.; Pirko, C.; Brahmbhatt, K.A.; Van Buren, G.; Carter, S.; Thompson, A.; Grogan, R.H.; Suliburk, J.; Eberlin, L.S. Clinical Translation and Evaluation of a Handheld and Biocompatible Mass Spectrometry Probe for Surgical Use. Clin. Chem. 2021 Sep 1, 67(9), 1271-1280. https://doi.org/10.1093/clinchem/hvab098

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