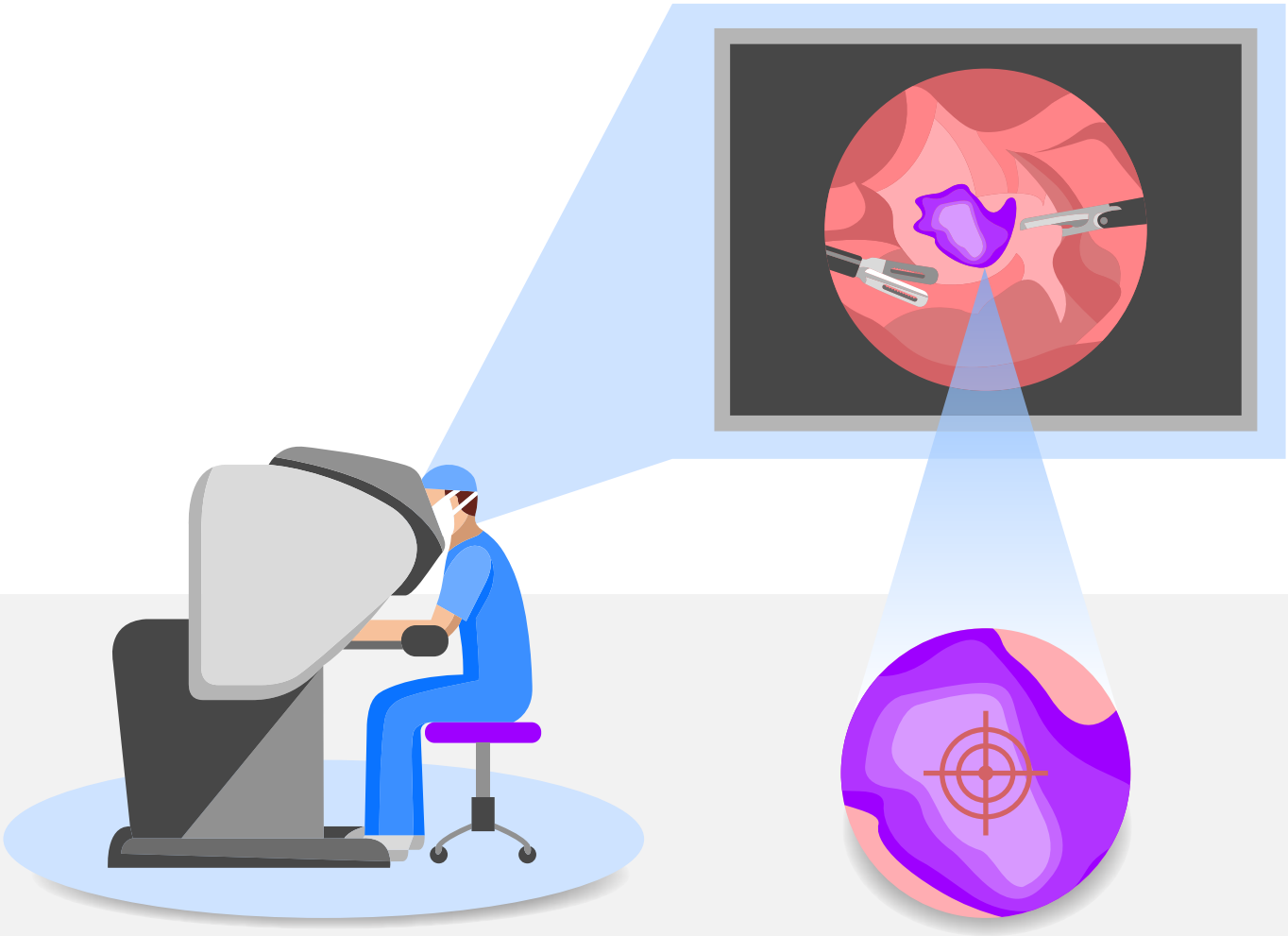
sagentia

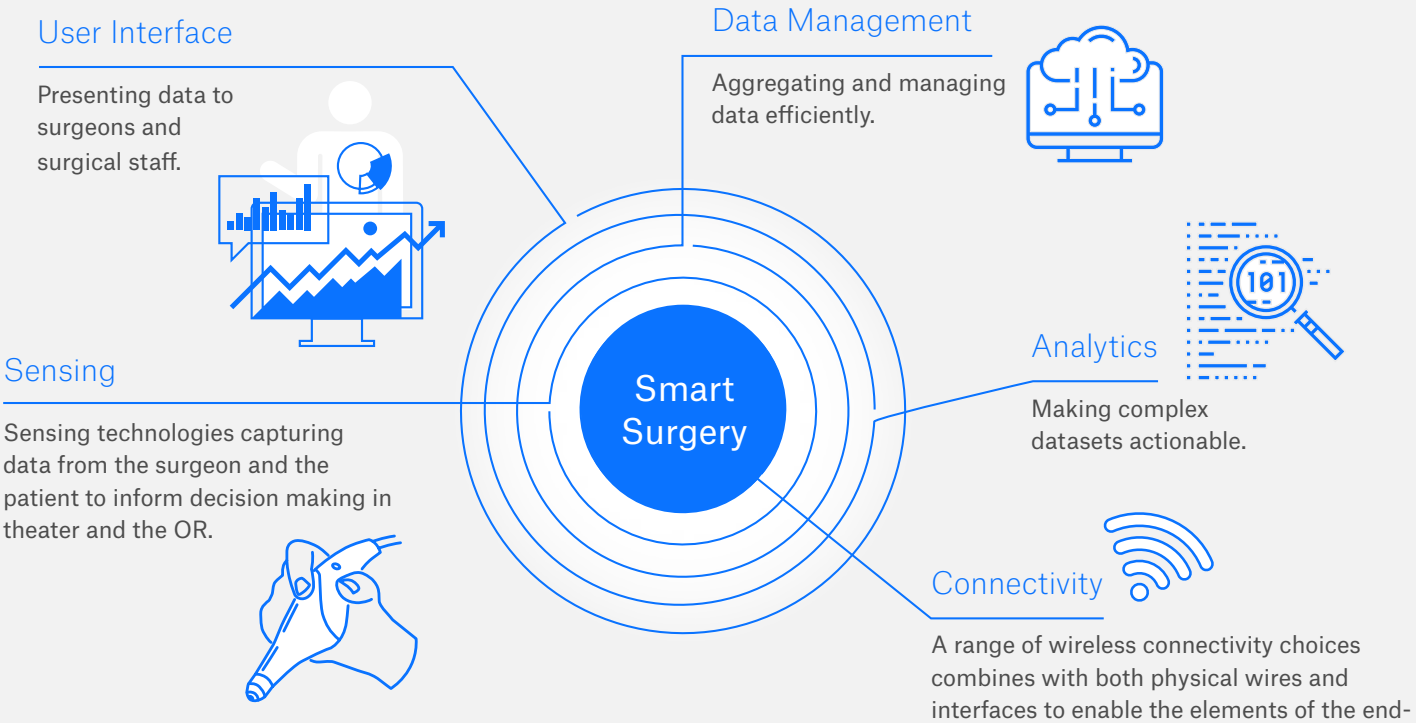


Transforming oncology surgery with label-free technologies

Thore Bücking and Rachel Smith from Sagentia's Applied Science Team explore the potential role of label-free sensing in the smart surgery phenomenon. Exempt from the regulatory and clinical burdens affecting biomarkers, these technologies could unlock new possibilities for oncology surgery.

sagentia

Smart surgery plays a lead role in the new era of intelligent medical technology. It provides surgeons with real-time information to improve decision-making in the operating room and offers benefits across the care continuum. Figure 1 outlines key enabling technologies that are powering the smart surgery revolution.



Label-free sensing technologies have much potential to drive progress in smart surgery, especially in the field of oncology surgery. Marker free imaging technologies avoid the need to tag tumours using markers, which is beneficial because it avoids the regulatory and clinical burden of introducing new markers to the market.

Oncology surgery

A primary goal of oncology surgery is the complete removal of diseased tissue. For more than a century, intraoperative diagnosis of tissue was performed by pathologists using light microscopy on frozen sections of removed tissue to identify morphological changes associated with cancer [1]. This technique had significant limitations, ranging from patients spending extended time under general anaesthesia to inaccuracies caused by technical limitations and subjective interpretation[1].

Today, real-time intraoperative sensing is used to detect tumour tissue, identify the location of critical structures and characterize tissue. In recent years, this has been achieved using biomarkers administered to the patient to allow easier sensing of the target. The most widely used example is Indocyanine Green (ICG), which is detected during surgery using fluorescence imaging.

New labels for the detection of specific tumour types are currently under development. However, the need for extensive testing related to safety and efficacy makes the regulatory and clinical route long, risky and expensive. Here, we look at three alternative approaches that can be used to sense cancerous tissue during surgery without the need for labels:

- Raman Spectroscopy based on the inelastic scattering of light, this technique can be used to identify the composition of tissue non-invasively
- Autofluorescence Spectroscopy by measuring the naturally occurring fluorescence of tissue, this analysis technique can be used to identify tissue composition and environment
- Mass Spectrometry measuring the mass-to-charge ratio of molecules in samples can be used to identify the composition of tissue invasively

Let's look at current developments, technical challenges and future potential for each of these technologies in relation to smart oncology surgery.

Raman Spectroscopy

The latest smart surgery applications and research

In recent years, various studies have demonstrated the capability of Raman spectroscopy to differentiate tumour from healthy tissue in vivo. Tumours that have been successfully identified include gliomas, glioblastoma multiforme and meningiomas amongst others (see ref [2] for a detailed review). While very promising, the technology has so far only been used intraoperatively on humans in a few pioneering studies.

Notable advances have been made by a research group in Montreal and its associated company ODS Medical:

Desroches *et al.* [3] demonstrated the capabilities of its hand-held system in a neurosurgery study involving 19 grade 2-4 glioma patients in targeted tissue surgical biopsy. They report a sensitivity of 80% and a specificity of 90% in differentiating dense cancer tissue from "non-diagnostic" tissue (280 samples).

Jermyn *et al.* achieved a sensitivity of 93% and specificity of 91% in identifying tissue with cancerous cells present (17 patients, 161 samples) [5]. They also report an increase in sensitivity and specificity to 100% and 93% respectively when including spectral information acquired with intrinsic fluorescence spectroscopy (15 patients, 230 samples) [4].

Also worth noting is Vancouver-based Verisante Technology Inc. It was the first to develop clinical hand-held Raman probes and initiated a human trial for intraoperative brain tumour detection in 2015. However, the company appears to have since moved in a different direction.

The studies highlighted above focus on the use of hand-held real-time diagnostic devices, capable of differentiating tissue in situ. Such devices typically consist of a fibreoptic bundle in combination with filter and focusing optics at the distal end. Optical components like these can withstand temperatures of surgical autoclave sterilization, meaning they can be engineered into reusable devices. Additionally, the relative simplicity on the distal side (passive, optical components) translates into a lightweight and small handpiece which should meet the demands of complex surgical procedures.

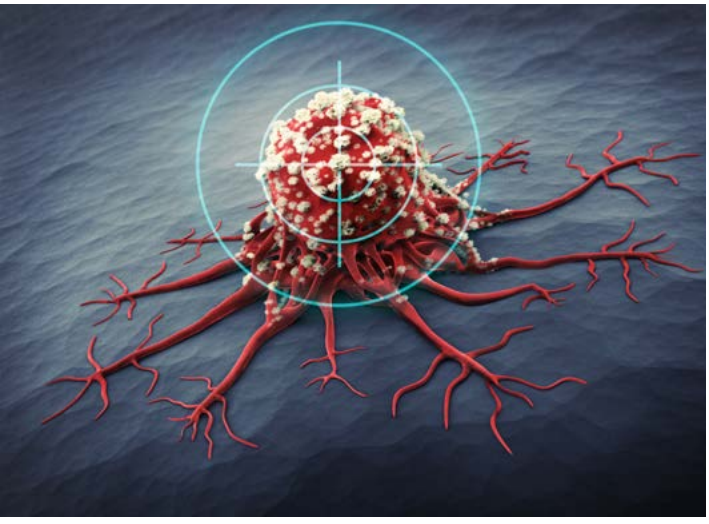
Technology challenges

The technical challenge with Raman spectroscopy for tissue classification in surgery is two-fold:

1. Only a small fraction of the detected light has undergone inelastic scattering. Typically, less than one in a million scattering events is due to Raman scattering, the rest being due to Rayleigh scattering, which does not cause a wavelength shift

2. In practice, the sensor interrogates a large range of molecules and the detected signal is the average of all their contributions. This complicates differentiation of fingerprints of different tissues as they largely consist of similar matter

Nevertheless, advances in laser technology, optical filters and detector sensitivity unlock new possibilities. Together with innovation in sensor arrangement and advanced processing and classification algorithms, this technique is becoming more viable for use within diagnostic tools for the operating room.



What's next

We see two outstanding issues which need to be addressed to bring Raman spectroscopy to maturity in surgical applications.

1. In vivo studies have based their success on machine learning techniques, but the training and testing data sets were very small. Typical machine learning training data sets for binary classification are in the range of thousands of samples even for relatively simple classifications. Performance can often degrade in larger test populations. For example, the optical MelaFind melanoma detection system was first reported as having a specificity of 85% (246 samples) [6], but a subsequent larger study (1,612 samples) found the system had a specificity of only 9.9% [7].
2. A fundamental problem with machine learning is lack of transparency. The system provides a clinician with a binary answer, but the reason for it is not apparent. To convince clinicians and regulatory bodies that algorithm outputs are trustworthy, they must also be explicable. (See our insight piece "Acting smarter with data" for more details).

Significant research is underway to make machine learning more transparent and it is only a question of time before we see adequate growth in training data sets. For example, the University of Nottingham has initiated a study to assess Fast Raman spectroscopy intraoperatively with 600 subjects undergoing Mohs surgery for skin cancer (NCT03482622). Rather than using a handheld device to provide point-measurements, the group intends to use a custom Raman micro-spectroscopy imaging system, which provides 2D images of excised tissue [8].

Tissue autofluorescence spectroscopy

The latest smart surgery applications and research

Autofluorescence monitoring is a keen area of interest for intraoperative analysis, especially in oncology. It detects the natural fluorescence that occurs when intracellular and extracellular endogenous fluorophores of tissue are excited by light at a suitable wavelength [9]. So, in contrast to the use of fluorescent probes, this technique does not require the administration of a label.

Endogenous fluorophores have much potential as intrinsic biomarkers for real-time intraoperative characterization since they are highly sensitive to minute chemical changes in their surroundings. In recent years, various studies have demonstrated the ability of tissue autofluorescence to distinguish tumour tissue from normal tissue in several organs. The technology's effectiveness has been demonstrated in various ex vivo samples (including on tissue excised during surgery).

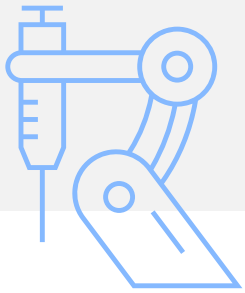
Tissue autofluorescence analysis has been integrated into the minimally invasive da Vinci surgical robot, providing real-time guidance with tissue diagnostic information [10]. A group at the University of California used this system for real-time inspection of oral cancers during transoral robotic surgery in swine and human patients. The same group has combined autofluorescence with ultrasound in a catheter for improved imaging of plaques in coronary arteries. These studies demonstrate the exciting applications of autofluorescence analysis in surgery.

Technology challenges

While the technology holds promise, *in vivo* use remains a challenge. Tissue components, such as red blood cells, collagen and lipofuscin have strong fluorescence, making it difficult to distinguish between the relevant tumorous signal and the tissue background. Fluorescence intensity is also dependent on the endogenous fluorophore concentration, which can be very low in peripheral tumour volumes and in tumour margins.

Additionally, distinguishing regions through simple emission wavelength analysis may not provide sufficient differentiation. More technically complex fluorescent lifetime measurements are often required to provide good specificity. Technical limitations typically constrain fluorescent lifetime measurements to spot measurement, rather than field imaging.

With equipment costs running to thousands of dollars and equipment set-ups comprising computers, multiple display monitors and illumination sources, another key challenge is the cost and size of the systems. What's more, as time-resolved fluorescence is currently a point measurement, the user must scan a probe over the tissue region, and overlay the results on image data to track moving tissue in real time.



What's next

Recent studies have attempted to address these limitations. Haidar and co-workers quantitatively measured fluorescence lifetime to improve imaging at tumour margins (due to the very low concentrations of fluorophores). The decay time of the fluorescence signal is dependent on environmental conditions, such as pH, temperature and structural changes, but not fluorophore concentration. This allowed the development of a scoring system to map tumours in brain tissue samples [11].

In a recent study, only modest specificity and sensitivity of 83% and 75% respectively were achieved [12]. Notingher and co-workers were able to enhance the sensitivity to 95% by combining autofluorescence and Raman spectroscopy in what they called 'multimodal spectral histopathology' [13]. As discussed earlier, Raman spectroscopy has been used to diagnose cancers with high sensitivity and specificity [14]. However, Raman spectroscopy is comparatively slow and is thus ill-suited to image larger areas with the necessary accuracy within the limited timeframe for diagnosis in vivo.

Notingher and co-workers used autofluorescence images to guide the Raman measurements to achieve high spatial and spectral information with small and large breast cancer tissue surfaces, achieving a specificity of 82% and sensitivity of 95% [13]. This has not yet been performed under real-time, intraoperative conditions, but only on surgical margin specimens.

Mass spectrometry

The latest smart surgery applications and research

In the last decade, intraoperative mass spectrometric (MS) tools have gained increasing attention. Two types of surgical tools have emerged, for sampling and diagnostics. Sampling tools allow real-time analysis while the tumour is being removed, whereas diagnostic tools locate the tumour allowing the surgeon to perform incision and removal.

There are two promising technologies in the first category: the iKnife (using rapid evaporative ionization MS "REIMS") and the picosecond infrared (PIR) lasers (PIRL) scalpel [15-16].

The iKnife was developed by scientists at Imperial College London and converts molecular constituents into charged gaseous particles (ions) using a surgical tool. The overall analysis time for aerosol transfer, MS analysis and data classification is 0.5-2s [17]. This gives the surgeon close-to-real-time feedback and orientation to resection margins. It has also achieved good accuracy in *ex vivo* analysis of liver, lung, colon (94.4% accuracy) and breast carcinoma (95.8 % accuracy) [18-19].

However, the iKnife requires a blade with a hot surface to allow evaporation to occur, which damages and thermally ablates surrounding tissue. This makes it impossible to confirm that the tumour has been fully removed by histological investigation [1]. A further drawback is the relatively low resolution provided, due to the blade width of 4mm, which could cause false negatives for the presence of tumours [1].

The current size and cost of this device precludes its use in many clinical settings, while mobility issues limit the ease with which it can be moved between operating rooms.

The PIRL scalpel is a comparable sampling tool. Here a PIR laser is used as an alternative to mechanical surgical tools. It provides high energy intensity in a confined area, leading to superheating and local tissue ablation, with minimal energy transfer to the surrounding area [16].

In addition to tissue ablation and rapid molecular detection, the laser has the ability to function as a cutting instrument. It has the added benefit of not damaging cells adjacent to the cutting site, resulting in minimal scarring [20]. The PIRL scalpel thus has a greater level of resolution in comparison to the iKnife (200-200 µm compared to 4 mm) but this higher resolution comes at the expense of sampling time (5-10s per spot compared to <2 s for the iKnife [16, 17]. Ideally, this timeframe limitation should be resolved prior to use in tissue screening analysis during surgery.

Technology challenges

The main challenge hindering the use of mass spectrometry techniques in smart oncology surgery relates to the trade-off between sampling time and m/z resolution. Rapid sampling is essential to enable shorter surgery, which minimizes damage and risk of infection. Yet resolution is also essential to ensure the whole tumour is excised. Another limiting factor – as with many such devices – is the cost and size of equipment, which impacts mobility and uptake.

What's next

Improvements to the iKnife and PIRL scalpel are one area of focus. However, additional developments in this space include desorption electrospray ionization (DESI)-MS and the MasSpec pen. These techniques can analyze tissue surface without damaging the cells.

DESI is so far the most extensively used ambient ionization technique and has high potential as an intraoperative real-time diagnostic tool for the future.

The MasSpec pen is a disposable, handheld probe currently in development, that uses ambient ionization MS [21]. The analysis is non-invasive, unlike the other methods discussed, and non-destructive (requiring only a droplet of water and no pressure), allowing further diagnostic analysis like histology.

A syringe pump delivers a small water droplet (4-10 µl) to the sampling probe, which dissolves and extracts the tissue surface molecules. After 3s of gentle physical contact with the tissue surface, the water droplet is transported to a mass spectrometer, which measures the levels of lipids, proteins and metabolites. A machine learning algorithm is then used to diagnose the probability of the presence of cancer in the tissue. This whole analysis takes ~10s.

The MasSpec pen, like DESI-MS, is purely diagnostic and has no cutting abilities. However, there is potential to harness the surface scanning abilities, non-invasiveness and low tissue damage as an added component to other devices where these are limiting factors. For example, the technology could be integrated with a cutting instrument so readings could be taken prior to a cutting event, without requiring the surgeon to change instrument.

Conclusions

Raman spectroscopy, autofluorescence spectroscopy and mass spectrometry offer much potential. They could play an important role improving oncology surgical outcomes and operation times. And the devices that leverage them are coming closer and closer to fulfilling this promise.

Raman and autofluorescence spectroscopy are optical and offer rapid, label-free diagnostic sensing in the operating room, without the need for a physical biopsy of the sample. Both are highly sensitive to minor chemical changes within the sample, but Raman scattering produces weaker signals than autofluorescence. This results in a slower acquisition time for a sufficiently strong signal. During surgery, an acquisition time of seconds, not minutes to hours, is needed.

Equally, autofluorescence has challenges to overcome prior to use intraoperatively. Tissue components produce a strong fluorescence signal, making it difficult to distinguish between tumorous tissue and background noise.

Current mass spectrometric tools have the advantage of being combined with a surgical cutting tool, such as the iKnife or PIRL scalpel. This allows rapid analysis while surgery is in progress, using a simple "point and shoot" approach for the surgeon. However, again there is a trade-off between sampling time and achieving sufficient m/z resolution.

Development challenges remain and the sampling time, portability, footprint, data analytics, clinical data set requirements and human acceptance of the data need to be considered in future devices. Interpreting gathered spectra can be complex and algorithms are required to understand the data generated then implement a simple system for the surgeon to easily distinguish between normal and tumorous tissue.

Cost is also a major factor – these technologies are currently expensive, which makes implementation in hospital operating rooms difficult. However, there are opportunities for future simplification and cost reduction.

Additional technologies are also being investigated, such as diffuse reflectance spectroscopy (DRS), optical emission spectroscopy (OES) [22] and electrical impedance spectroscopy (EIS) [23]. Each has its own strengths and weaknesses in achieving rapid tissue differentiation during surgery.

We will continue to monitor these developments for their potential to enable commercially viable smart surgery systems. This is a dynamic and exciting field which will continue to evolve at pace.

sagentia

References

[1] Lorena Hänel, Marcel Kwiatkowski, Laura Heikaus and Hartmut Schlüter. 'Mass spectrometry-based intraoperative tumour diagnostics', Future Sci OA, 5, no. 3 (March 2019): FSO373. doi: 10.4155/fsoa-2018-0087

[2] Lakomkin, Nikita, and Constantinos G. Hadjipanayis. 'The Use of Spectroscopy Handheld Tools in Brain Tumor Surgery: Current Evidence and Techniques'. Frontiers in Surgery 6 (29 May 2019): 30. https://doi.org/10.3389/fsurg.2019.00030.

[3] Desroches, Joannie, Michael Jermyn, Michael Pinto, Fabien Picot, Marie-Andrée Tremblay, Sami Obaid, Eric Marple, et al. 'A New Method Using Raman Spectroscopy for in Vivo Targeted Brain Cancer Tissue Biopsy'. Scientific Reports 8, no. 1 (December 2018): 1792. https://doi.org/10.1038/s41598-018-20233-3.

[4] Jermyn, Michael, Jeanne Mercier, Kelly Aubertin, Joannie Desroches, Kirk Urmey, Jason Karamchandiani, Eric Marple, Marie-Christine Guiot, Frederic Leblond, and Kevin Petrecca. 'Highly Accurate Detection of Cancer In Situ with Intraoperative, Label-Free, Multimodal Optical Spectroscopy'. Cancer Research 77, no. 14 (15 July 2017): 3942–50. https://doi.org/10.1158/0008-5472.CAN-17-0668.

[5] Jermyn, Michael, Kelvin Mok, Jeanne Mercier, Joannie Desroches, Julien Pichette, Karl Saint-Arnaud, Liane Bernstein, Marie-Christine Guiot, Kevin Petrecca, and Frederic Leblond. 'Intraoperative Brain Cancer Detection with Raman Spectroscopy in Humans'. Science Translational Medicine 7, no. 274 (11 February 2015): 274ra19-274ra19. https://doi.org/10.1126/scitranslmed.aaa2384.

[6] Elbaum, Marek, Alfred W. Kopf, Harold S. Rabinovitz, Richard G.B. Langley, Hideko Kamino, Martin C. Mihm, Arthur J. Sober, et al. 'Automatic Differentiation of Melanoma from Melanocytic Nevi with Multispectral Digital Dermoscopy: A Feasibility Study'. Journal of the American Academy of Dermatology 44, no. 2 (February 2001): 207–18. https://doi.org/10.1067/mjd.2001.110395.

[7] Monheit, Gary, Armand B. Cognetta, Laura Ferris, Harold Rabinovitz, Kenneth Gross, Mary Martini, James M. Grichnik, et al. 'The Performance of MelaFind: A Prospective Multicenter Study'. Archives of Dermatology 147, no. 2 (1 February 2011): 188. https://doi.org/10.1001/archdermatol.2010.302.

[8] Kenny, Kong, Manon Bourbousson, Sandeep Varma, David Baldwin, Irshad Soomro, and Ioan Notingher. 'Integrated Raman Microscopy and Auto-Fluorescence Imaging for Fast Tumour Diagnosis during Cancer Surgery'. In Biomedical Optics 2016, JW4A.7. Fort Lauderdale, Florida: OSA, 2016. https://doi.org/10.1364/CANCER.2016.JW4A.7.

[9] Monici M. 'Cell and Tissue Autofluorescence Research and Diagnostic Applications', Biotechnol. Annu. Rev., 11 (2005): 227-56. doi: 10.1016/S1387-2656(05)11007-2

[10] Gorpas D, Phipps J, Bec J, Ma D, Dochow S, Yankelevich D, Sorger J, Popp J, Bewley A, Gandour-Edwards R, Marcu L, and Farwell D. 'Autofluorescence lifetime augmented reality as a means for real-time robotic surgery guidance in human patients', Sci Rep., 9 (2019): 1187. doi: 10.1038/s41598-018-37237-8

[11] Poulon F, Pallud J, Varlet P, Zanello M, Chretien F, Dezamis E, Abi-Lahoud G, Nataf F, Turak B, Devaux B & Haidar D. 'Real-time Brain Tumor imaging with endogenous fluorophores: a diagnosis proof-of-concept study on fresh human samples', Scientific Reports, 8 (2018): 14888. https://www.nature.com/articles/s41598-018-33134-2?draft=marketing

[12] Coda S, Thompson A, Kennedy G, Roche K, Ayaru L, Bansi D, Stamp G, Thillainayagam A, French P & Dunsby S. 'Fluorescence lifetime spectroscopy of tissue autofluorescence in normal and diseased colon measured ex vivo using a fiber-optic probe', Biomed. Opt. Express, 5, no. 2 (2014): 515–538. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3920881/

[13] Shipp D, Rakha E, Koloydenko A, Macmillan R, Ellis I and Notingher I, 'Intra-operative spectroscopic assessment of surgical margins during breast conserving surgery', Breast Cancer Res., 20, no. 69 (2018). https://breast-cancer-research.biomedcentral.com/articles/10.1186/s13058-018-1002-2

[14] Pradipta A, Tanei T, Morimoto K, Shimazu K, Noguchi S and Tanaka K, 'Emerging Technologies for Real-Time Intraoperative Margin Assessment in Future Breast-Conserving Surgery', Advanced Science, 7, 1901519 (2020). https://onlinelibrary.wiley.com/doi/epdf/10.1002/advs.201901519

[15] Alexander J, Gildea L, Balog J, et al. 'A novel methodology for in vivo endoscopic phenotyping of colorectal cancer based on real-time analysis of the mucosal lipidome: a prospective observational study of the iKnife', Surg. Endosc., 31, no. 3, (2017): 1361–1370.

[16] Amini-Nik S, Kraemer D, Cowan ML, et al., 'Ultrafast mid-IR laser scalpel: protein signals of the fundamental limits to minimally invasive surgery', PloS One., 5, no. 9, (2010):e13053.

[17 St John ER, Balog J, Mckenzie JS, et al. 'Rapid evaporative ionisation mass spectrometry of electrosurgical vapours for the identification of breast pathology: towards an intelligent knife for breast cancer surgery', Breast Cancer Res., 19, no. 1 (2017): 59.

[18] Jaafar H. 'Intra-operative frozen section consultation: concepts, applications and limitations', Malaysian J. Med. Sci., 13, no. 1 (2006): 4–12.

[19] Van Den Brekel MW, Lodder WL, Stel HV, Bloemena E, Leemans CR, Van Der Waal I. 'Observer variation in the histopathologic assessment of extranodal tumor spread in lymph node metastases in the neck', Head Neck., 34, no. 6 (2012): 840–845.

[20] Petersen H, Tavakoli F, Kruber S, et al. 'Comparative study of wound healing in rat skin following incision with a novel picosecond infrared laser (PIRL) and different surgical modalities', Lasers Surg. Med., 48, no. 4 (2016):385–391.

[21] MasSpec Pen, URL: https://www.masspecpen.com/

[22] Spether D, Scharpf M, Hennenlotter J, Schwentner C, Neugebauer A, Nüßle D, Fischer K, Zappe H, Stenzl A, Fend F, Seifert A, and Enderle M, "Real-time tissue differentiation based on optical emission spectroscopy for guided electrosurgical tumor resection," Biomed. Opt. Express 6 (2015): 1419-1428

[23] Hillary SL, Brown BH, Brown NJ et al. Use of Electrical Impedance Spectroscopy for Intraoperative Tissue Differentiation During Thyroid and Parathyroid Surgery. World J Surg 44 2020: 479–485. https://doi.org/10.1007/s00268-019-05169-7

About Sagentia

Sagentia is a global science, product and technology development company. Our mission is to help companies maximize the value of their investments in R&D. We partner with clients in the medical, consumer, industrial and food & beverage sectors to help them understand the technology and market landscape, decide their future strategy, solve the complex science and technology challenges and deliver commercially successful products.

Sagentia employs over 150 scientists, engineers and market experts and is a Science Group company. Science Group provides independent advisory and leading-edge product development services focused on science and technology initiatives. It has ten offices globally, two UK-based dedicated R&D innovation centers and more than 400 employees. Other Science Group companies include OTM Consulting, Oakland Innovation, Leatherhead Food Research, TSG Consulting and Frontier Smart Technologies.

For further information visit us at:

www.sagentia.com
or email info@sagentia.com
www.sciencegroup.com

sagentia

Sagentia Ltd
Harston Mill
Harston
Cambridge
CB22 7GG
UK

Sagentia Ltd
First Floor
17 Waterloo Place
London
SW1Y 4AR
UK

Sagentia Inc
One Beacon Street
15th floor, Suite 1500
Boston
MA 02108
USA