Intraoperative virtual histology

Stimulated Raman spectroscopy combined with machine learning generates histological images for the rapid diagnosis and classification of brain tumours.

Carlyn A. Figueiredo and James T. Rutka

The diagnosis and management of brain tumours depend on a number of factors, which include clinical findings, neuroimaging studies depicting tumour location and accurate neuropathological diagnosis. Intraoperative diagnosis of the neuropathology of a tumour sample is critical in determining the best possible therapeutic strategy employed by the neurosurgeon. Traditional methods of histopathological diagnosis involve sample preparation of tissue sections that follow well-established fixation and staining protocols. This method has been 'tried and true' for decades, and has yielded good results in accurately identifying tumour tissue. However, the practice has had limitations. For example, the size of the intraoperative tumour sample, whether it is a needle biopsy or a core sample, can influence the diagnostic power, particularly when considering the phenotypic heterogeneity of brain tumours. Additionally, rare brain tumours may be misclassified, given their challenging and rarely seen histopathological features. Moreover, the technical aspects of tumour sample processing may introduce artefacts. Reducing these sources of error is critical in maximizing the chances of an accurate tumour diagnosis, and could theoretically be accomplished by intraoperative diagnostic tools, applied to fresh specimens, that lead to reliable readouts and less processing time.

In this respect, stimulated Raman spectroscopy (SRS) has emerged as a technique to accurately distinguish features of different tissues on the basis of nucleotide, protein, and lipid spectral profiles. SRS can be used to track DNA properties to aid the rapid diagnosis of human skin cancer and to produce images comparable to those of traditional haematoxylin and eosin (H&E) preparations. Also, properties of SRS images, such as protein/lipid ratio, axonal density and cellularity, can be used to identify infiltrative tumour cells in brain tissue, with accuracy greater than 99%. Now, by taking advantage of machine learning, Daniel Orringer and colleagues report in Nature Biomedical Engineering the first implementation of SRS microscopy for the rapid generation of histological images for the diagnosis and sub-classification of brain tumours. The method — termed stimulated Raman histology (SRH) — uses an SRS microscope engineered to function within the operating room.

Typically, two Raman shifts (~2,845 cm⁻¹ and ~2,930 cm⁻¹) form the basis of SRS imaging. An image of the 2,845 cm⁻¹ shift highlights lipids in the tissue section, and a subtraction of this from the 2,930 cm⁻¹ shift shows the architecture of proteins and nucleotides. These characteristics have been sufficient to provide clear images of tumour infiltration and tumour boundaries by assigning two different colours to the images. Orringer and colleagues used instead pseudo-colouring to produce H&E-like images that appear much more familiar to pathologists.

Orringer and co-authors used SRH to scan samples from 101 neurosurgical patients, paying special attention to the unique cellular and morphological properties of normal and tumour tissues. Reference tissue to examine the histological features of normal brain parenchyma was taken from the anterior temporal lobe following temporal lobectomy. SRH showed many unique characteristics, including axons, lipofuscin granules and macrophage infiltration, which can sometimes affect diagnosis. For gliomas, SRH showed clear differences between low- and high-grade tumours, in particular in the degree of cellularity, shape of the nucleus, microvascular proliferation and perinuclear halos. SRH was also useful in determining tissues of non-glial origin, namely meningioma and lymphoma.

It is well known that glial tumours can be histopathologically heterogeneous such that a single small biopsy of a glioma may not be sufficient to provide a clear picture for diagnosis. In one low-grade glioma sample, Orringer and colleagues demonstrate the histological pattern of diffuse cells with high cytoplasmic density, but also a field of hypercellularity and mitotically active cells showing anaplastic features. Because of the rapid turnaround time of SRH, the method allows the neuropathologist to survey different parts of a tumour so as...
to obtain a more comprehensive view of the different cell types and cell behaviour, thereby influencing the diagnosis and the intraoperative neurosurgical treatment plan.

Given that the traditional protocol of histopathological diagnosis involves H&E staining of frozen tissue sections, it was essential for Orringer and co-authors to verify that the quality of the images generated by SRH was comparable with previous methods in providing neuropathologists with similar, if not enhanced, information. The authors performed quantitative analysis of the diagnoses made by three neuropathologists that examined the SRH data. Interestingly, the neuropathologists could distinguish lesional from non-lesional tissue with 98% accuracy, and glial from non-glial tumours with 100% accuracy. Furthermore, a near-perfect concordance between the traditional H&E method of diagnosis and SRH was observed in predicting the final diagnosis, which was 92% accurate.

However, to a certain degree the accuracy can be confounded by the experience of the neuropathologist and the quality of images produced. Yet by collating a large volume of SRH data with different tumour sub-types, it is possible to establish a database with which samples can be compared to predict possible diagnoses. Orringer and co-authors used a machine-learning system known as multilayer perceptron (MLP) to accomplish this. To produce a library of SRH histopathologies, the authors employed a ‘leave-one-out’ approach to include all samples investigated from 30 patients, except the one under investigation. This resulted in a total of 12,879 fields of view (FOV) classified into four different categories: non-lesional tumour, low-grade glial tumour, high-grade glial tumour and non-glial tumour. The MLP demonstrated outstanding predictive capability, with 100% accuracy for lesional versus non-lesional tissue, and 90% accuracy for diagnostic class. Because of the phenotypic heterogeneity of glial tumours, and because a combination of different FOV would yield a probability based on the majority consensus, it is expected that minor morphological details may still be missed.

Owing to the ease of use and fast processing times as short as 2.5 minutes, SRH as an intraoperative diagnostic tool has promise for clinical implementation. The advantage of obtaining real-time feedback on fresh tissue makes SRH a viable asset for the neurosurgeon during surgery, and can help dictate the best options for patients after resection. However, the automated MLP system does not completely discount the necessity of an experienced neuropathologist. There still exists the potential to miss rare tissue properties and tumours that may not be covered by the SRH dataset for the MLP. Additionally, clinical information and intraoperative findings are relevant towards making an accurate diagnosis in a holistic format, which may be challenging to accomplish with an automated system. However, SRH offers significant advantages over existing tissue-sectioning protocols, as it can streamline diagnosis until additional information can be gathered.

Conventional Raman spectroscopy using an intraoperative Raman probe allows for live surveying of specimen samples prior to resection through contact with the tissue and for a spectral readout that can be correlated to cancerous or normal tissue. In the future, combining SRS and a probe-based system could provide diagnostic information and influence surgical decision-making. Although the utility and safety of SRS and SRH need to be validated by clinical trials, it is evident that Raman spectroscopy shows great potential for improving diagnostic and therapeutic power in neurosurgery.

Carlyn A. Figueiredo is at the Labatt Brain Tumour Research Centre, Hospital for Sick Children, 686 Bay Street, Toronto, Ontario M5G 1X8, Canada. James T. Rutka is in the Department of Laboratory Medicine and Pathobiology, University of Toronto, 1 King’s College Circle, Toronto, Ontario M5S 1A8, Canada. E-mail: james.rutka@sickkids.ca

References