HORIBA Jobin Yvon

Raman Division, 231 Rue de Lille, 59650 Villeneuve d'Ascq, Lille, France
Tel: +33 (0) 3 20 59 18 00, Fax: +33 (0) 3 20 59 18 08
E-mail: info@jobinyvon.com, Website: www.jobinyvon.com

Spectroscopic imaging for the life sciences–more than just a pretty picture

Dr Simon FitzGerald[a] and Dr Gwénaëlle le Bourdon[b]
[a]HORIBA Jobin Yvon Ltd, 2 Dalston Gardens, Stanmore, Middlesex, HA7 1BQ, UK
[b]HORIBA Jobin Yvon SAS, 231 Rue de Lille, Villeneuve d'Ascq, Lille, France

Introduction

It has long been a major goal of scientists to understand the many complex processes taking place within living organisms, ranging from microscopic species such as bacteria and viruses at one extreme, through to the human at the other. Biomedical understanding has grown at an amazing pace, developing deeper knowledge of living processes and providing ever more effective disease treatments, but the thirst for knowledge continues. As scientific techniques and instrumentation develop at an equally fast pace, it is perhaps no surprise that biologists are venturing into new realms for their research. One such area which is proving extremely promising is that of micro-spectroscopic imaging.

Microscopic imaging is a technique which has long been key to biological research, stemming back to the first investigations with optical microscopes. More recent experiments such as fluorescence imaging, confocal laser scanning microscopy, electron microscopy and atomic force microscopy have certainly enhanced imaging capabilities, but there is still something that these techniques lack. That is, characterisation of a sample's elemental/molecular composition.

This is where spectroscopic techniques come in. For example, laser Raman spectroscopy probes the interactions of individual bond vibrations with light, resulting in an information rich, environment sensitive spectrum which gives detailed insight into the chemical composition of the sample. Another contrasting technique is x-ray fluorescence (XRF), which provides *elemental* information through atomic interactions with an x-ray beam.

In recent years, both techniques have been developed into fast micro-analytical methods—for example, bench top Raman microscope systems allow a spatial resolution of just 1 µm to be achieved, whilst x-ray beams with diameters down to 10 µm are now routinely available on bench top XRF systems.

Through the use of automated sample movement, it is easily possible to harness these micro-spectroscopic tools for imaging—stepping or scanning the sample allows a complex hyperspectral data array to be built up, comprising a full spectrum at each and every XY position on the sample. From this, it is a small step to generate detailed spectral images showing clear chemical or elemental distribution across the sample.

The importance of such non-destructive analysis for the life sciences is fast being realised, and already spectroscopic imaging is providing answers for skin care, cancer diagnosis, drug–cell interactions, bacteriology, dentistry etc.

Zinc mediation in ulcer healing

Micro-XRF is ideally suited to investigations of elemental accretions in tissue. In addition to the high spatial resolution possible, a key consideration for such work is the ability to analyse at atmospheric pressure—even for the biologically important light elements such as sodium, magnesium and aluminium. Older technology often requires harsh vacuum conditions, so that biological tissues containing large amounts of water are quickly dehydrated and subsequently destroyed.

Dr Takeshi Ohtsuka of Kyoto Prefectural University of Medicine, Japan, has studied the role of zinc in healing processes of gastric ulcers using micro-XRF, by comparing ulcer regions with and without application of zinc containing medication.

Mapping experiments allowed the zinc distribution within rat gastric tissue to

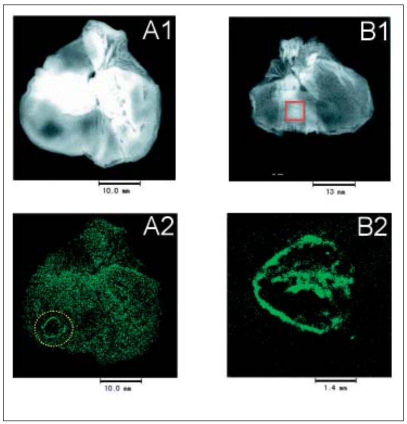

Figure 1. (1) X-ray transmission and (2) zinc XRF mapped images from (A) untreated and (B) treated rat stomach tissue. For A, the entire stomach was mapped, with the ulcer region highlighted by the yellow circle in A2. The red box in B1 illustrates the small ulcer area chosen for mapping and represented in B2.

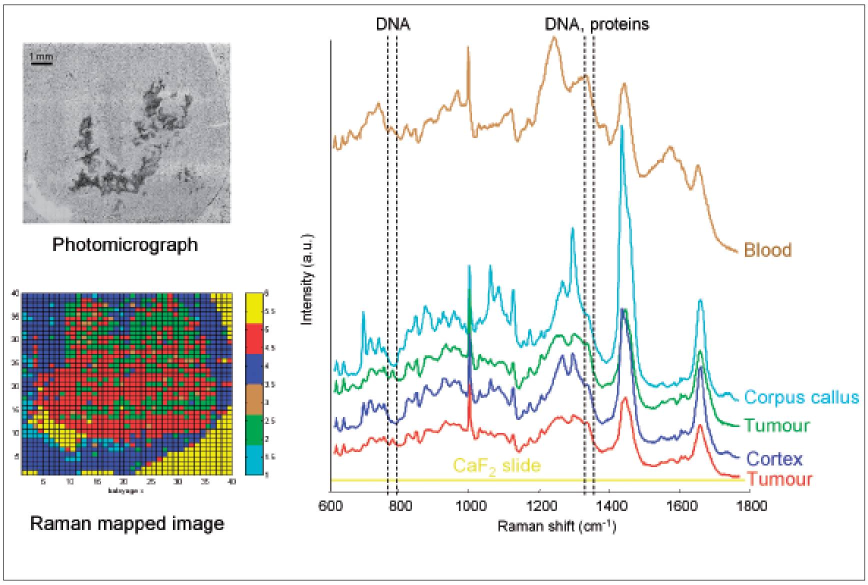

Figure 2. Photomicrograph and Raman mapped image of a rat brain tissue section, with coloured regions corresponding to the offset spectra shown on the right.

be quickly characterised, particularly in the marginal regions of the ulcer. Figure 1 clearly shows increased zinc concentration at the edges of and within the ulcer region. That this is observed in the untreated tissue suggests the body will use natural sources of zinc to aid healing of the ulcer. In tissue from a rat treated with zinc containing medication, the accumulation of the element at the ulcer region is increased, and is evident not only in the marginal regions, but within the ulcerated tissue too. From these experiments the positive role of zinc in ulcer treatment can be confirmed.

Cancer diagnosis

Whereas XRF provides elemental information, the *molecular* information yielded by Raman allows complex biochemistry within tissues to be probed, giving information on the many typical species found within cells, such as lipids, proteins, DNA, RNA and carbohydrates. The exact balance of these constituents of course depends upon tissue type and health state, and so by probing in this manner, Raman is ideally suited to gaining further understanding of such matters.

Within cancer research and treatment, traditional methods of diagnosis are based on histopathological staining and a (trained) human eye, but such methods can often only distinguish between clearly distinct health states. With Raman, the detailed chemical information means that more subtle distinctions between tissue states can be found, and already there are many encouraging reports of the diagnostic success of Raman—as a goal, it is hoped that such analysis will allow not only a very fast and reproducible decision on whether tissue is cancerous or not, but also a clear indication of a tumour's development stage and its malignancy.

Professor Manfait and co-workers at the Université de Reims, France, have been key in harnessing the power of Raman micro-spectroscopy for the life sciences, and recent work has focussed on diagnosis of glioma tumours. These are a particularly aggressive form of brain cancer, spreading quickly and often evading traditional surgical techniques for removal.

Figure 2 illustrates results from mapped imaging of an unstained rat brain tissue section. Different tissue types are shown in the pseudo-colour Raman map, corresponding to both healthy (corpus callus, cortex, and blood) and diseased (tumoral) tissue, as well as the calcium fluoride slide.

The red tumoral zone correlates directly to histopathological observations with H/E staining, and clearly illustrates the extent of developed tumour within the tissue. The green tumoral zone is not picked up through histopathological observations, and is tentatively assigned to an early development stage of the tumour. The spectra indicate the high DNA content of the cancerous tissue, caused by the fast growing, proliferative nature of the cells.

Conclusions

As these examples show micro-spectroscopic techniques are fast becoming invaluable tools for the life sciences, allowing researchers to move closer to a complete understanding of living processes. The images resulting from techniques such as Raman or XRF are more than just pretty pictures—they provide the scientist with a new dimension of information, based upon real biochemistry and composition. The possibilities are unlimited.

Reference

1. N. Amharref, S. Dukic, A. Beljebbar, L. Venteo, A. Leveques, M. Pluot, M. Manfait, *Discriminating healthy from tumor tissue on rat glioma by spectroscopy imaging, and characterization of spectroscopic modifications induced by antitumoral treatment* in vitro *and* ex vivo, presented at "DataSpec 2005" congress, Reims, France.