Brochure: BR015(EN)-01-A

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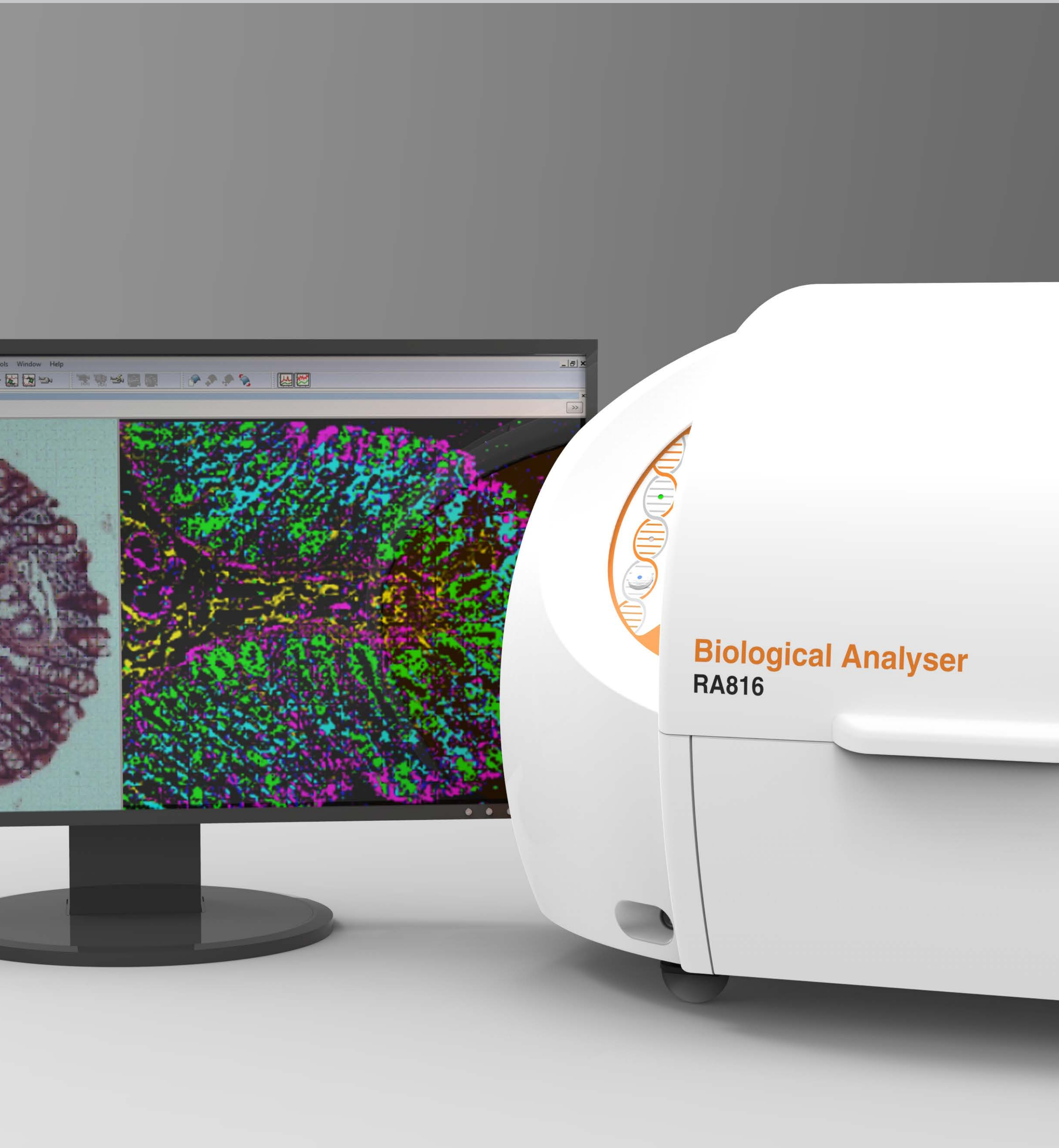
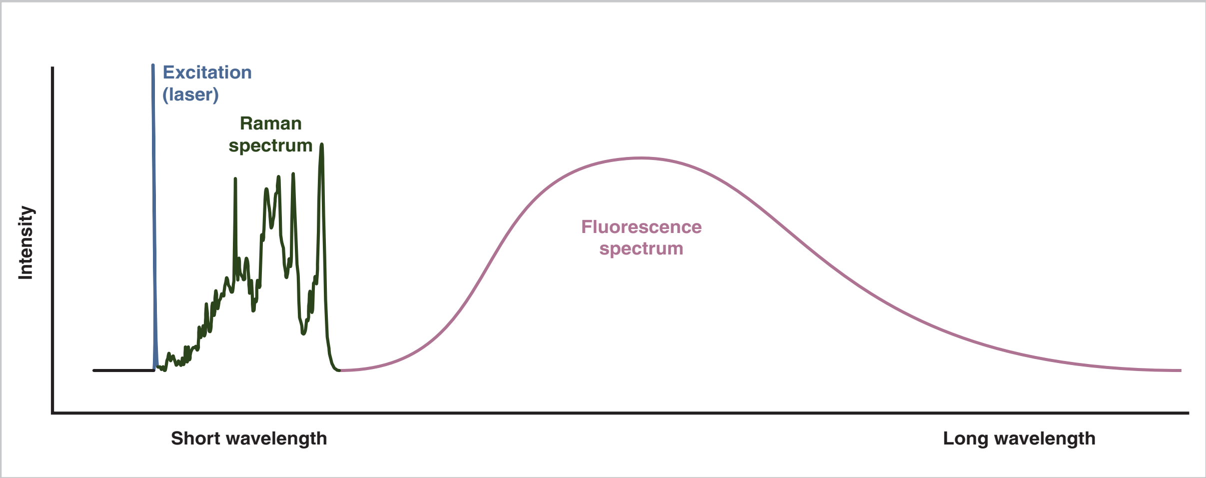
Biological analysis using Raman spectroscopy and imaging



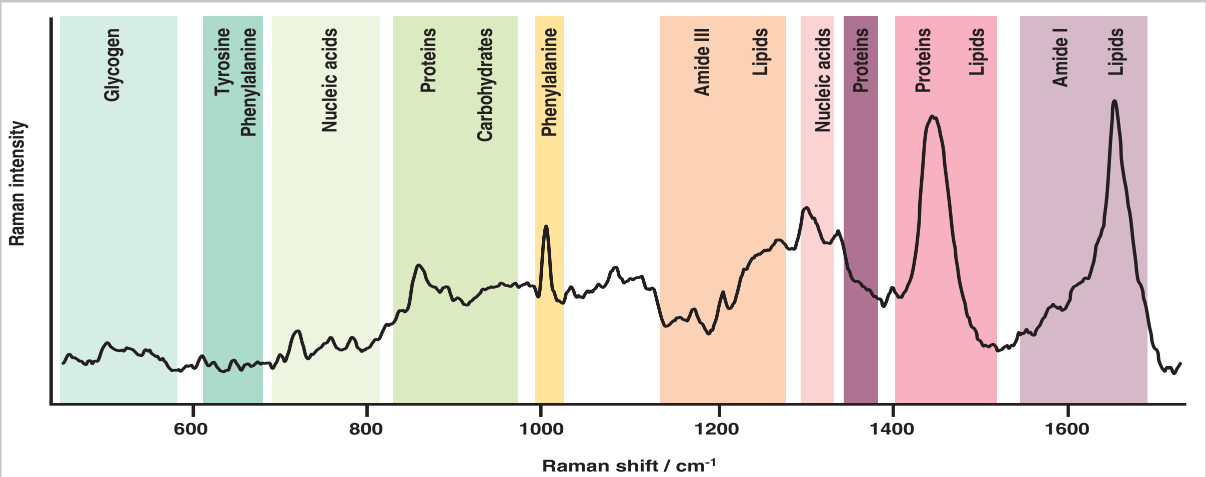
What is Raman spectroscopic imaging?

Raman imaging is an optical technique that can rapidly provide chemical and structural information about biological specimens. It is non-invasive and has high spatial resolution, enabling you to study biological systems in fine detail. The information generated can be both qualitative (identification of biochemical constituents) and quantitative (the amounts of each constituent present). This makes it a very powerful tool for biological studies.

A Raman system illuminates the sample with a laser and analyses the scattered light. This produces spectra that are unique to each chemical species. Renishaw Raman systems can also scan the sample under the laser to produce 2D and 3D images from multiple points to reveal the distribution and concentration of biomolecules.



Raman spectra occur closer in wavelength to the excitation source than fluoresence spectra. Although Raman spectra normally cover a much smaller range of wavelengths, they contain much more detailed structural and chemical information (diagram not to scale).



A Raman spectrum from a typical tissue, demonstrating the wealth of information obtained from a single measurement (single pixel).

How can Raman imaging help me?

The benefits of Raman imaging for the study of biological material include:

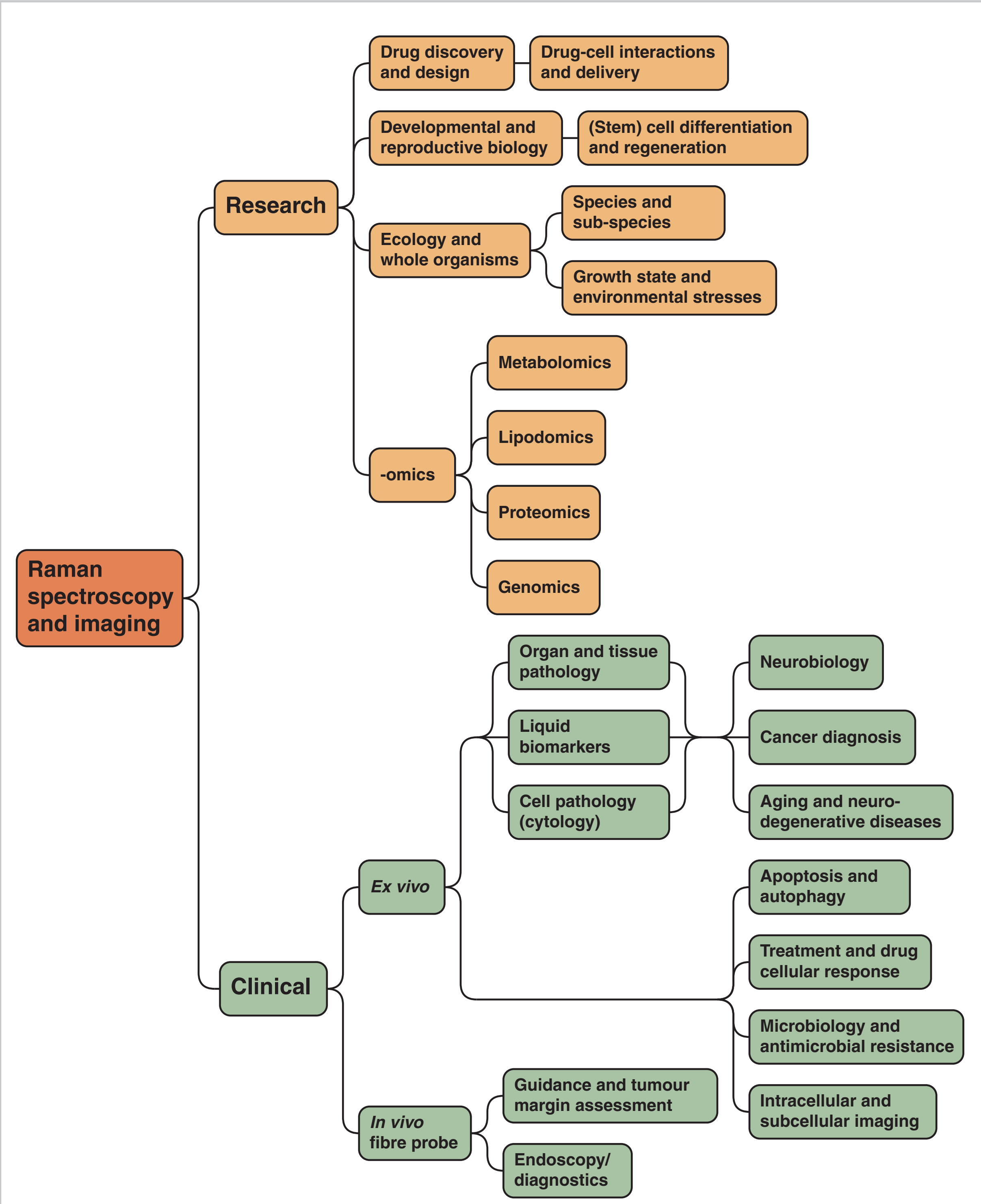
- Analysis is objective: it is related directly to molecular structure, not inferred by other means, such as tags
- You do not need to label or use contrast agents or stains, saving time and avoiding sample modification
- It is non-destructive and non-invasive, enabling later analysis by other bio-analytical techniques
- It can be used both *in vivo* and *ex vivo*
- Analysis is rapid: individual spectra are acquired in seconds
- Each analysed point on the sample contains a wealth of biochemical information
- It provides micrometre-scale spatial information, enabling you to analyse tumour margins and determine structure

All the molecules being illuminated by the laser contribute to the Raman spectrum. These normally include structural and metabolic proteins, nucleic acids, different phospholipids, carbohydrates, and other small molecules. However you can computer process the spectrum to extract quantitative information on each constituent present in a complex mixture.

Renishaw Raman systems can also acquire multiple spectra and process them to generate false colour images (akin to stained sections), or also use the multiple spectra as a basis for diagnostic models for disease prediction.

Renishaw, expert in Raman imaging, produces a range of instruments used in many application areas.

Raman imaging in research and clinical applications

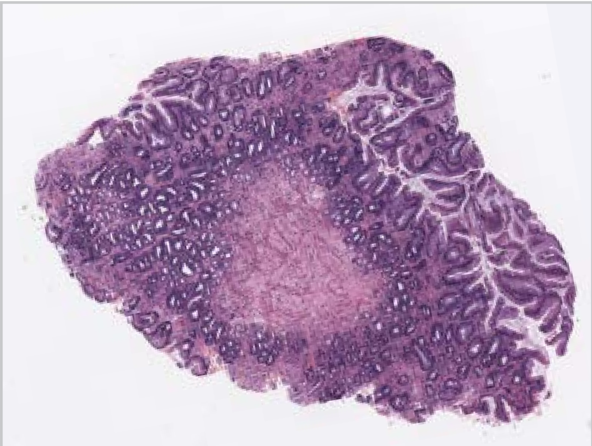


Researchers have used Raman imaging in many fields of biology.

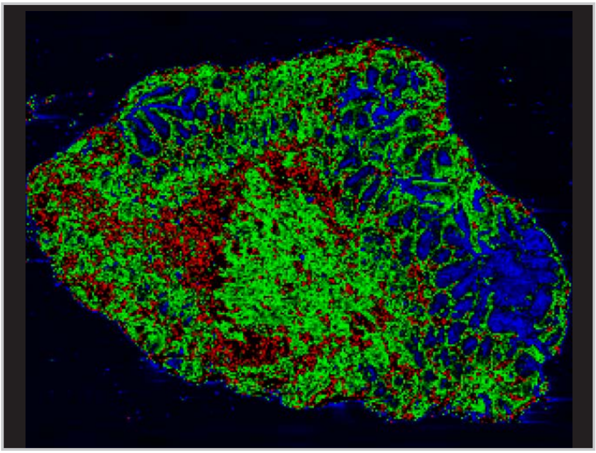
Research

Raman imaging provides high spatial resolution chemical and structural information. It does this non-invasively and rapidly (in seconds).

It can make qualitiative measurements of biochemical constituents, and also generate quantitative information.



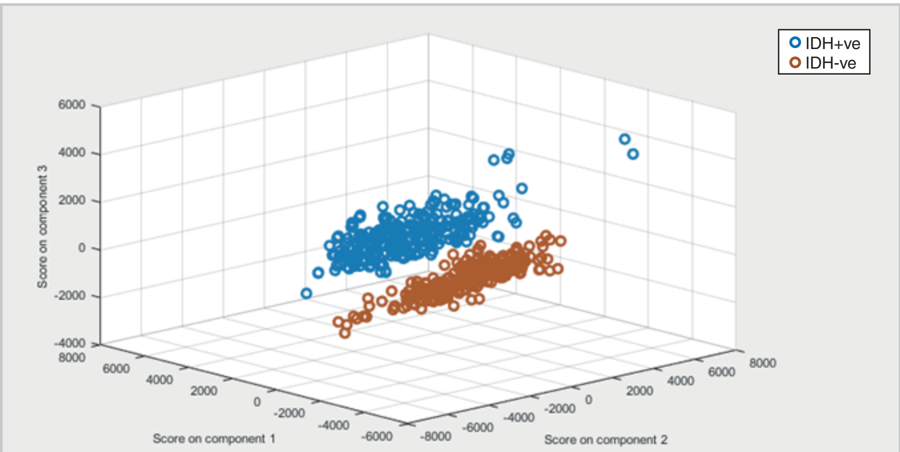
H&E stained image of Barrett's oesophagus tissue.



Corresponding Raman biochemical image from an unstained tissue section. The colours indicate different tissue types, such as: mucosal cells; fibrous tissue; goblet cells; and glandular tissue.

In clinical diagnosis

Raman imaging has the potential to integrate into existing patient care pathways. You can use chemical and structural information about different pathology states to develop classification models (with these you can classify unknown clinical samples). This information, alongside other clinical and molecular diagnostic data, can aid patient treatment planning.



Raman spectroscopic data from brain glioma samples, plotted to show how you can distinguish the status of the IDH genomic mutation. The two clusters are well separated, showing that Raman spectral data can be used to discriminate between different pathology states.

Example applications: cellular biology (cytology)

Whole cell live imaging

Raman imaging is ideal for live cell studies. It can reveal intracellular (subcellular) composition under normal physiological conditions, without the need for labelling. From receptor/target identification to cellular drug metabolism, it has applications throughout drug discovery as well as monitoring cellular drug uptake and distribution. And, because the Raman signal from water is very weak, you can also study molecules and cells in aqueous environments.

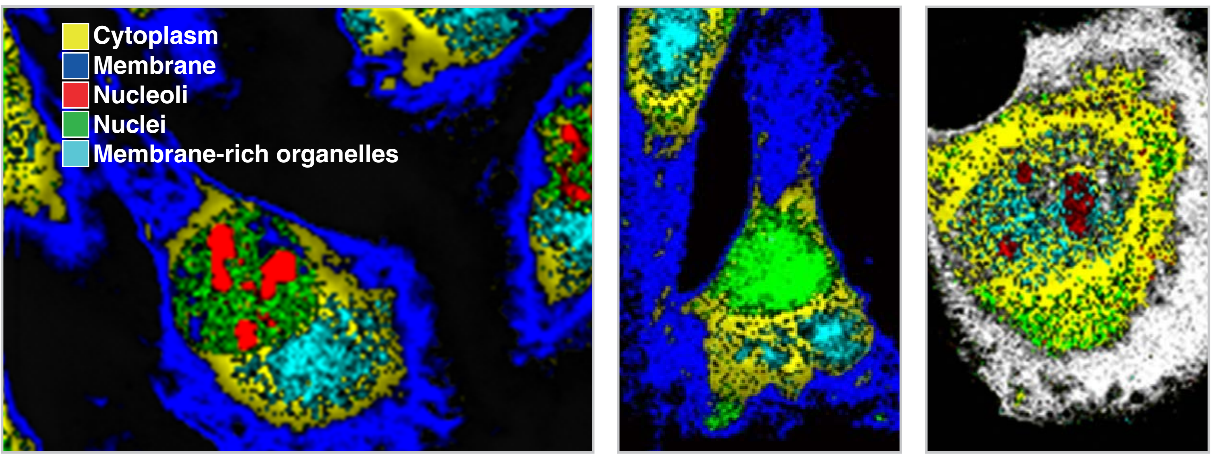
Microbiology

It is important to detect and identify bacteria in clinical medicine, food safety and contamination control. Patients with a bacterial infection can greatly benefit from the early diagnosis and identification of medically relevant microorganisms.

Current cultivation and test methods are time consuming (taking several days) and are laborious. Raman imaging offers a new rapid and accurate approach for bacterial identification.

Key Raman imaging literature

1. In situ characterisation of living cells by Raman spectroscopy. Notingher, Verrier, Romanska, Bishop, Polak, Hench. *Spectroscopy*. 2002;16(2):43-51.
2. In vitro biophysical, microspectroscopic and cytotoxic evaluation of metastatic and non-metastatic cancer cells in responses to anti-cancer drug. Li, Xiao, Harihar, Welch, Vargis, Zhou. *Anal Methods*. 2015;7(24):10162-10169.
3. A Raman spectroscopic study of cell response to clinical doses of ionizing radiation. Harder, Matthews, Isabelle, Brolo, Lum, Jirasek. *Appl Spectrosc.* 2015;69(2):193-204.
4. Influence of culture media on microbial fingerprints using raman spectroscopy. Mlynáriková, Samek, Bernatová, … Holá. *Sensors (Switzerland).* 2015;15(11):29635-29647.
5. Discrimination of Bacteria Using Surface-Enhanced Raman Spectroscopy. Jarvis, Goodacre. *Anal Chem.* 2004;76(1):40-47.
6. Investigating Microbial (Micro)colony Heterogeneity by Vibrational Spectroscopy. Choo-Smith, Maquelin, Van Vreeswijk, … Endtz. *Appl Environ Microbiol.* 2001;67(4):1461-1469.
7. The subcellular distribution of small molecules: From pharmacokinetics to synthetic biology. Zheng, Tsai, Zhang, Rosania. *Mol Pharm.* 2011;8(5):1619-1628.
8. Microfluidic chip for non-invasive analysis of tumor cells interaction with anti-cancer drug doxorubicin by AFM and Raman spectroscopy. Zhang, Xiao, Li, Qi, Zhou. *Biomicrofluidics*. 2018;12(2).



A comparison of the size and distribution of organelles and biomolecules in normal and autophagic MG-63 cells: normal (left), autophagic (centre) and apoptotic (right).

Example applications: protein and nucleic acids (proteomics and genomics)

Protein conformational structure

Raman imaging can be used to analyse both crystalline and dissolved proteins, and study conformations and local environments.

You can study samples in aqueous solutions, monitoring subtle conformational changes as they occur, whilst preserving biological activity.

Transmembrane and cell surface proteins

Raman imaging enables you to study the structural and functional characteristics of proteins on and within cell membranes. This gives you a better understanding of cellular dynamics, cellular processes and the signals vital for biological cell function and survival.

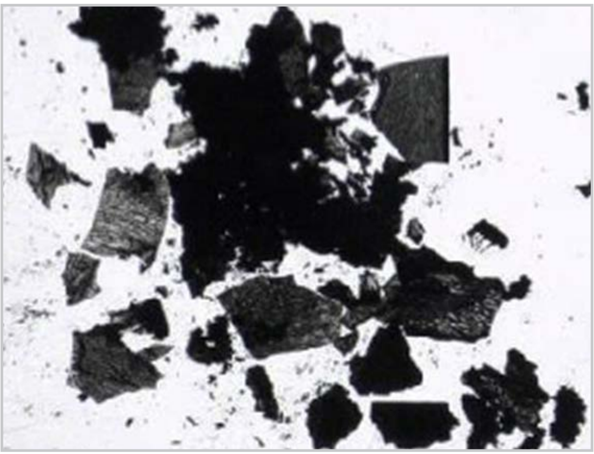
Abnormalities in cellular signalling—even at the level of cell surface proteins—cause many diseases. Imaging cells with Raman spectroscopy can identify these biochemical and structural changes. You can do this under normal physiological conditions, without the need of chemical labels or stains that might affect the processes occurring.

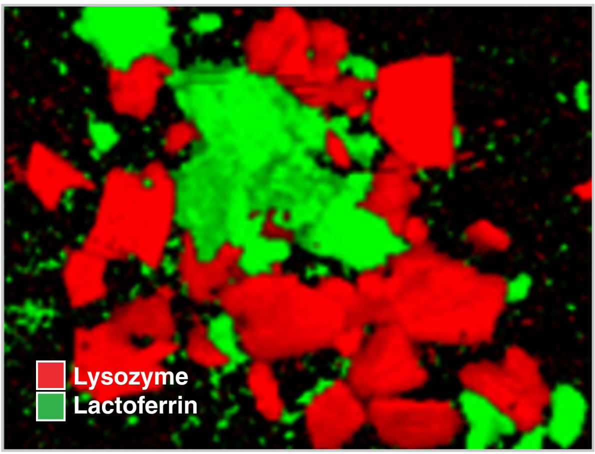
DNA sequencing and quantification

Diagnostic tests that require the quantification of nucleic acids use the polymerase chain reaction (PCR) and fluorescent agents. Raman imaging can provide information about DNA content without the need for dye binding. You can also study DNA packing state, conformation, composition, as well as the nucleotide sequence of the DNA and RNA.

Key Raman imaging literature

1. Use of surface-enhanced Raman spectroscopy for the detection of human integrins. Chowdhury, Gant, Trache, Baldwin, Meininger, Coté. *J Biomed Opt.* 2006;11(2):024004.
2. Raman spectroscopy for DNA quantification in cell nucleus. Okotrub, Surovtsev, Semeshin, Omelyanchuk. *Cytom Part A.* 2015;87(1):68-73.
3. Monitoring the glycosylation status of proteins using raman spectroscopy. Brewster, Ashton, Goodacre. *Anal Chem.* 2011;83(15):6074-6081.
4. Investigation into the protein composition of human tear fluid using centrifugal filters and drop coating deposition Raman spectroscopy. Filik, Stone. *J Raman Spectrosc.* 2009;40(2):218-224.
5. Detecting chemically modified DNA bases using surface-enhanced raman spectroscopy. Barhoumi, Halas. *J Phys Chem Lett.* 2011;2(24):3118-3123.
6. Characterisation of DNA methylation status using spectroscopy (mid-IR versus Raman) with multivariate analysis. Kelly, Najand, Martin. *J Biophotonics.* 2011;4(5):345-354.





Images of dry proteins taken with white light microscopy (left) and Raman imaging (right). The white light image cannot distinguish between the proteins, but the Raman image reveals the distribution of lysozyme (red) and lactoferrin (green).

Example applications: tissue and biofluid diagnostics and pathology

With Raman spectroscopic imaging you can biochemically assess cell and tissue function non-destructively, without needing labels.

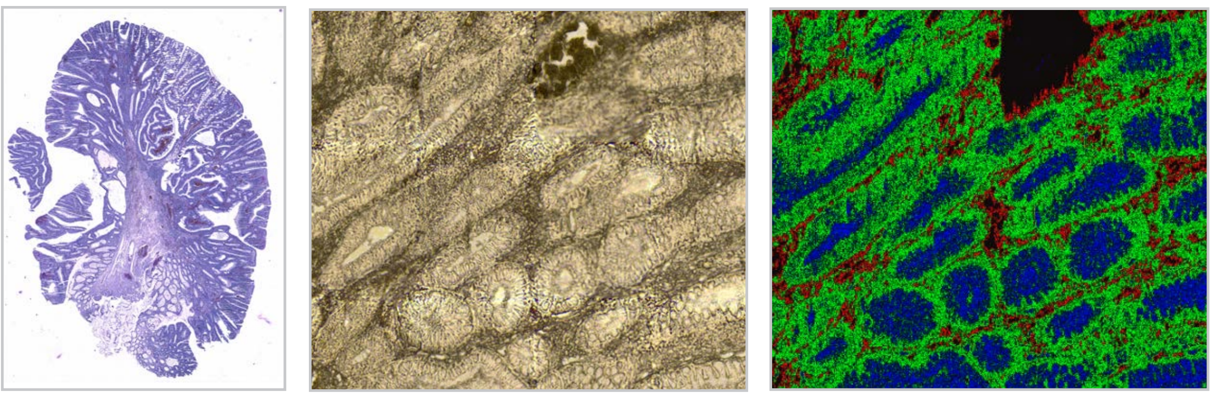
The research community has undertaken clinical studies and established that Raman spectroscopy can distinguish diseased from healthy tissue, and also diagnose the grade of cancer and disease type. This has been determined for various tissue types including brain, oesophagus, colon, breast and prostate.

This work has been performed on bulk biopsy tissue and slide-mounted tissue sections, demonstrating flexibility of sample type.

Disease screening programmes are mostly dependent on invasive procedures. These involve removing tissue during a surgical procedure, and result in a degree of morbidity to patients. It is less invasive to use biofluids—such as blood, urine, cerebrospinal fluids (CSF) or saliva—for screening. Researchers have shown Raman spectroscopy is a promising technique for diagnosing disease using biofluids.

Key Raman imaging literature

1. Raman Spectroscopy to Diagnose Alzheimer's Disease and Dementia with Lewy Bodies in Blood. Paraskevaidi, Morais, Halliwell, … Martin. *ACS Chem Neurosci.* 2018;9(11):2786-2794.
2. Raman spectroscopy for medical diagnostics - From *in-vitro* biofluid assays to *in-vivo* cancer detection. Kong, Kendall, Stone, Notingher. *Adv Drug Deliv Rev.* 2015;89:121-134.
3. Raman spectroscopy analysis of the biochemical characteristics of molecules associated with the malignant transformation of gastric mucosa. Chen, Dai, Zhou, Liu, Zhang, Peng. *PLoS One.* 2014;9(4).
4. Potential of non-invasive esophagus cancer detection based on urine surface-enhanced Raman spectroscopy. Huang, Wang, Chen, … Chen. *Laser Phys Lett.* 2014;11(11).
5. Identification of regions of normal grey matter and white matter from pathologic glioblastoma and necrosis in frozen sections using Raman imaging. Kast, Auner, Yurgelevic, … Kalkanis. *J Neurooncol.* 2015;125(2):287-295.
6. Discriminant analysis of Raman spectra for body fluid identification for forensic purposes. Sikirzhytski, Virkler, Lednev. *Sensors.* 2010;10(4):2869-2884.
7. Developing and understanding biofluid vibrational spectroscopy: A critical review. Baker, Hussain, Lovergne, … Sockalingum. *Chem Soc Rev.* 2016;45(7):1803-1818.



Analysing GI tract tissue. H&E stained tissue images are shown: overview (left); zoomed-in (centre). The Raman image of the zoomed-in region (right), from an adjacent unstained tissue section, reveals the distribution of bio-molecules.

Example applications: bone, teeth and cartilage

The health and quality of human bone tissue is strongly dependent on its chemical composition. You need information about composition to study bone development, aging, pathology, microstructure, and changes in mechanical function. The mineral/organic matrix ratio is a direct measure of mineral content and can be determined using Raman imaging.

Mineral crystallinity is related to mineral maturity. It affects mineral crystallite size, mineral maturity, and the amount of substitution into the bone apatitic mineral lattice. Carbonate and phosphate Raman features provide mineral crystallinity information, an important factor in bone fracture initiation, propagation and the effect of tensile and compressive stresses.

Researchers have studied osteoarthritis with Raman imaging. You can investigate bone mineralisation, cartilage matrix vesicles and changes in cartilage composition by changes in their Raman spectra.

Raman imaging can also be used to study dental tissue. It can characterise natural tissues' dentin and enamel components, and mineral phase in synthetic compounds. Research on healthy and carious teeth has shown differences indicative of demineralization-induced alterations.

Some examples of the use of Raman imaging in dental health and oral hard tissue pathologies include: dental caries; periodontitis; amelogenesis imperfecta; dentinogenesis imperfecta; and fluorosis. It has also identified oral microbial flora that potentially affect oral hygiene and health.

Key Raman imaging literature

1. Raman Spectroscopy of Bone and Cartilage. Morris. In: *Emerging Raman Applications and Techniques in Biomedical and Pharmaceutical Fields.* ; 2010:347-364.
2. Raman assessment of bone quality. Morris, Mandair. In: *Clinical Orthopaedics and Related Research.* Vol 469. ; 2011:2160-2169.
3. Micrometer-Sized Magnesium Whitlockite Crystals in Micropetrosis of Bisphosphonate-Exposed Human Alveolar Bone. Shah, Lee, Tedesco, … Palmquist. *Nano Lett.* 2017;17(10):6210-6216.
4. Evaluation of degenerative changes in articular cartilage of osteoarthritis by Raman spectroscopy. Oshima, Kiyomatsu, Miura, Ishimaru, Hino. In: Farkas, Nicolau, Leif, eds. *Imaging, Manipulation, and Analysis of Biomolecules, Cells, and Tissues XVI.* Vol 10497. SPIE; 2018:45.
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7. Applications of Raman spectroscopy in dentistry: Analysis of tooth structure. Ramakrishnaiah, Rehman, Basavarajappa, … Rehman. *Appl Spectrosc Rev.* 2015;50(4):332-350.
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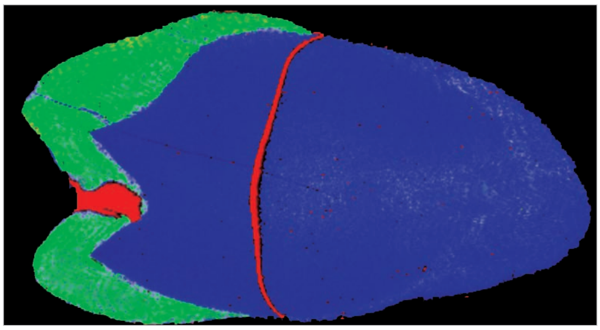
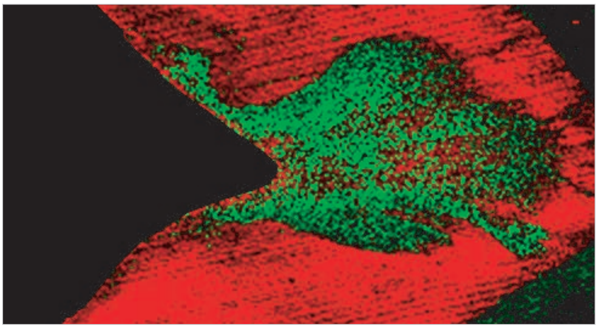


A Raman image of a complete tooth section, revealing the dentine region (blue), enamel (green) and areas of high fluorescence (red).



A Raman image of part of a tooth, showing the carious regions (green) in high detail.

Why choose Renishaw?

Renishaw is a world leader in Raman imaging instrumentation. It has over 25 years' experience supplying Raman systems to customers worldwide. It has a global network of scientists and engineers on-hand to provide expert product and technical support. Renishaw has extensive experience designing and developing Raman systems, providing solutions with superior performance. Our systems can transform biological studies and cell-drug interaction discovery and development.

Our products

Renishaw Raman systems combine the highest performance with ultra-fast operation. You can generate information-rich chemical images and see the composition and distribution of biochemical constituents in intricate detail. Coupled with Renishaw's powerful software our systems enable you to analyse a range of biological samples, generating both qualitative and quantifiable results.

Biological Analyser RA816

This system is designed exclusively for tissue and biofluid diagnostics and analysis. The RA816 is optimised for routine tissue analysis where speed, automation and ease of use are important. It is ideal for use in pathology laboratories that need to rapidly analyse multiple tissue samples.

inVia™ confocal Raman microscope

Renishaw's inVia is a fully configurable research-grade Raman microscope. Scientists worldwide trust inVia and its unparalleled flexibility. It can be upgraded, modified and customised, without compromising performance. Add accessories, lasers, fibre optic probes, or combine it with other analysis techniques. Whichever configuration you choose, you will have the most flexible and sensitive Raman microscope available.

Stories from some of our customers

Early cancer diagnosis: developing leading-edge biosensors

Singapore Bioimaging Consortium (SBIC), Singapore

The Singapore Bioimaging Consortium develops biosensor platforms specifically optimised for early stage cancer diagnosis. These platforms incorporate a research-grade Renishaw Raman microscope. Biomarker expression levels in early stage cancer can be particularly low, making detection and monitoring difficult. However they used an amplification technique (SERS - surface enhanced Raman scattering) to boost the Raman signal, giving improved detection levels.

This platform uses SERS nanotags (and antibodies) inside the core of hollow-core optical fibres for multiplex detection.

These bind to protein antigen biomarker targets from cancer cells, tissues and fluids (e.g. serological liver cancer biomarkers) and amplify their Raman signals, to give ultrasensitive detection.

For further details please see: www.renishaw.com/sbic

Quality checking of red blood cells (RBCs) in blood bags

Michael Smith Laboratories at the University of British Columbia in Vancouver, Canada

The Michael Smith Laboratories at the University of British Columbia in Vancouver, Canada, is pioneering the use of Raman imaging for monitoring biochemical changes and inter-donor variability in stored red blood cell (RBC) units.

The group collaborated with Canadian Blood Services, using Raman imaging to investigate chemical changes occuring during storage. They used an inVia confocal Raman microscope to analyse the bulk properties of blood with minimal interference from the surrounding bag material. They are investigating whether Raman imaging could be used as a quality check prior to transfusion.

The group has also used Raman imaging to characterise single cells and populations of cells *in vitro* as they respond to a variety of chemical and environmental stimuli. These stimuli include those triggering differentiation, autophagy, necrosis and apoptosis. The group also uses Raman imaging to analyse sub-cellular glycogen and nucleoli.

For further details please see: www.renishaw.com/rn067

Classification and genetic subtyping of gliomas (brain cancer)

Nuffield Department of Clinical Neuroscience, University of Oxford and Oxford Radcliffe Hospital, United Kingdom

Renishaw collaborated with scientists and clinicians at Oxford Radcliffe Hospital, using Raman imaging to classify gliomas from different pathological preparations by their genetic subtypes.

James Livermore, a Clinical Research Training Fellow, has shown how Renishaw's Biological Analyser can rapidly classify the genetic subtypes of glioma during surgery. This allows surgeons to tailor their surgical strategy based on the patient's specific tumour genetics.

For further details please see: www.renishaw.com/ra816

For more information about Renishaw Raman systems and their applications in the biological sciences field please contact us at raman@renishaw.com or discover more at www.renishaw.com/bio

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About Renishaw

Renishaw is an established world leader in engineering technologies, with a strong history of innovation in product development and manufacturing. Since its formation in 1973, the company has supplied leading-edge products that increase process productivity, improve product quality and deliver cost-effective automation solutions.

A worldwide network of subsidiary companies and distributors provides exceptional service and support for its customers.

Products include:

- Additive manufacturing and vacuum casting technologies for design, prototyping, and production applications
- Dental CAD/CAM scanning systems and supply of dental structures
- Encoder systems for high-accuracy linear, angle and rotary position feedback
- Fixturing for CMMs (co-ordinate measuring machines) and gauging systems
- Gauging systems for comparative measurement of machined parts
- Laser and ballbar systems for performance measurement and calibration of machines
- Medical devices for neurosurgical applications
- Probe systems and software for job set-up, tool setting and inspection on CNC machine tools
- Raman spectroscopy systems for non-destructive material analysis
- Sensor systems and software for measurement on CMMs
- Styli for CMM and machine tool probe applications

For worldwide contact details, visit www.renishaw.com/contact

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