

Impact of Raman Microscopy on Biomedical Research

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Raman spectroscopy is a vibrational technique complimentary to Infrared (IR) Absorption. They both provide information on materials via their molecular and crystalline vibrations. While IR spectroscopy has been used more widely, Raman has some distinctly different characteristics that provide advantages when making measurements on biological materials. For a start, water, while it does produce a spectrum, that spectrum is weak and does not present an overwhelming background as it does in the IR; since biological matrices are, by definition, aqueous, this is an enormous advantage. The second important difference lies in the focusing capability in microscopic sampling; the physical limitations to microscopic sampling depend on the wavelength of the radiation. Raman spectra are excited by lasers with wavelengths between 1064 and 244 nm which are 4 to 400 times shorter than the wavelengths probed by infrared absorption. An IR microscope can acquire spectral maps of histological tissue sections (hundreds of μm 's), but not cells (7-12 μm size range), certainly not organelles (tenths of μm 's) or bacterial micro-organisms ($\sim 1 \mu\text{m}$). In addition, The acquisition of Raman spectra are not limited to thin samples or to surface measurements; it is possible to probe below a surface if a sample is not opaque. For this article we have selected three topics where there is groundbreaking activity in Raman microscopy worldwide and where the implementation has potential to solve outstanding problems. Information on instrumentation innovations that are critical to some of this work (especially lab-on-a-chip) will be mentioned. The references, while not exhaustive, will provide the interested reader entry to the literature. The topics that have been selected are identification of bacterial micro-organisms, disease diagnosis, and Raman imaging.

Introduction

Raman spectroscopy is not a new technique, having been first described by C. V. Raman in 1928. In the early years Raman spectroscopy was used in the domain of a small number of advanced Physics labs. More recently Raman spectroscopy has developed into a useful and wide ranging non-invasive analytical tool, providing fast chemical identification and discrimination. The technique measures the interaction of monochromatic laser light with chemical bonds within the sample, providing rich spectral information on the sample's molecular and structural make up. Since it is a technique using light irradiation, it can be adapted for use on an optical microscope.

Raman spectroscopy and microscopy have long been of interest in biomedical research because of the wealth of information contained in the vibrational spectrum. The

spectra of proteins, lipids, nucleic acids, and carbohydrates are characteristically different, but different species in a given class are also differentiable from another in that class. There are many places where an interested researcher can get an introduction to Raman spectroscopy, but for biomedical applications, we can recommend a review article which has an extensive table listing Raman vibrational frequencies with assignments to the various classes of biological materials^[1].

Technical hurdles have prevented the utilization of Raman spectroscopy/microscopy in biomedical research until the last 20 years. A number of innovations have changed this so the application of the technology is explosive. In this article we will explore some areas that are quite active where Raman microscopy has the potential to make groundbreaking changes to the biomedical field.

Microbial Identification

The usual size of a bacterium is commensurate with the size of a focused laser beam ~ ca. 0.5–1.0 μm . This means that any spectrum generated by a focused beam is likely to represent the entire bacterial organism. As early as 1990 spectra of single cells were reported^[2], and subsequently spectra of single bacterial cells were published in 2000^[3] and 2004^[4]. In the 2000 publication by Schuster, et.al., it was shown that changes in chemical composition related to growth stage and granule storage were easily identified. Tentative band assignments were made, and seven different bacterial species were differentiated by the spectra in the 2004 reference. In addition Huang, et.al., also showed that it was possible to differentiate growth stage (phenotypic classification) of a given organism, and samples grown in a ^{13}C -enriched glucose carbon source produced identifiable red shifts of some of the bands reflecting incorporation of the heavier isotope in newly synthesized molecules of the bacteria. It will be clear that this ability to detect isotope shifts enables one to follow bacterial metabolism which has some very important applications.

For example, in 2007 Prof. Huang's group combined stable isotope probing (SIP) Raman spectroscopy and fluorescence *in situ* hybridization (FISH) to follow environmental degradation of isotopically labelled naphthalene^[5]. The importance of this work was the ability to both identify the naphthalene degradation, and to identify the bacteria that were responsible using the ^{13}C incorporation into the organisms. In fact, this work was done in groundwater, not laboratory preparations, in order to have access to bacteria that cannot be grown in culture media, which

is quite important because of the large percentage of organisms that cannot be cultured in the laboratory.

A further development from this group is "Raman-activated cell ejection" (RACE)^[6, 7]. In this scheme, cells flow through a microfluidic device where a Raman spectrum of a single cell can be recorded and used to decide whether to collect the cell and then use it for biological assay. The goal is to "link... gene expression to cell physiological states and phenotypes."

Identification of micro-organisms is clinically and environmentally relevant. In particular, the identification of strain as well as species of bacterium in the emergency room setting is important in order to select an antibiotic that will be effective against the patient's infection. Currently the method to determine the damaging bacterium is to culture for 2-3 days; the result will be the identification of both the species and strain. Until that information is available, a patient is often treated with a broad spectrum antibiotic in order to keep him/her alive; this actually increases the possibility of the development of resistant strains. The potential of Raman analysis to provide identification in hours can be the difference between life and death as well as minimizing the development of resistant strains. In order for Raman spectroscopy to be able to identify bacterial species and strain, a database of the spectra of relevant organisms has to be collected and made available. Spectral collection, etc. has to be standardized (eg., to include the age of a colony and the nutrition on which it grew) and multivariate algorithms have to be rigorously tested for positive results. Data bases have to be continuously updated to include new antibiotic-resistant strains. Numerous authors have

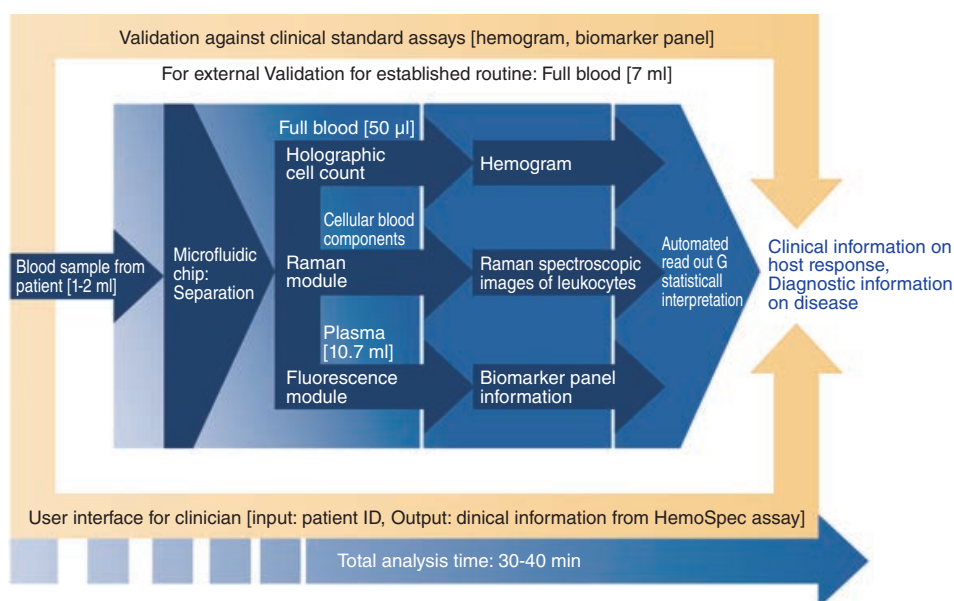


Figure 1 Scheme proposed by European Union for identifying infectious organism and preparing the best antibiotic defense. This figure is reproduced from <http://www.hemospec.eu/index.php/technology>.

discussed this potential^[8-12]. And the European Union has formed a consortium to address these needs (hemosp. eu). Figure 1 outlines their suggested protocol for identifying an organism from a minimum size blood sample.

The last citation above^[12] is a review article in the Journal of Raman Spectroscopy that appeared in 2015 and summarizes many applications in environmental samples, food contaminants, and medical care where the identification of an organism is of interest. In these discussions, there are some non-medical applications that have some relevance to human safety. They include the detection of bacterial bioterrorism agents, and food and water pathogens. While this material is of great interest, much of it is outside the scope of this short article, so, if interested, the reader is strongly advised to refer to the article directly.

In this review^[12] the authors describe multiple applications of Raman spectroscopy for identifying organisms with high sensitivity and specificity, sometimes in a high-throughput mode. It is important to recognize that all these measurements require extensive databases of reference spectra of micro-organisms and sophisticated multivariate algorithms. For example, a system was tested in hospital outbreaks of infection and found to be effective for “screening variability within a collection of isolates.” They also reviewed the application of SERS (surface enhanced Raman scattering) for rapid/high-throughput identification of bacteria by integrating Raman spectroscopy with a microfluidic device. In some instances, SERS methods were used to detect pathogen-specific nucleic acid sequences of pathogenic bacteria, fungi and viruses. Particular attention was paid to describing the detection of pathogens in body fluids (also without cultivation). The ones that were covered were urine, blood and serum, ascites (fluid from the abdominal cavity), sputum, cerebrospinal fluid, tears, and cervical fluid. In addition, the modes of infection of skin and nails were studied. Publications examining antibiotic interactions with bacteria were examined; for example, it was found that the Raman signature could differentiate bacteria that were sensitive to or resistant towards vancomycin. Another study differentiated bacteriostatic vs. bacteriocidal actions of an antibiotic by detecting Raman bands of DNA.

The last topic that these authors reviewed was the effectiveness of Raman spectroscopy to detect intracellular pathogens, and to differentiate the same pathogen inside of and outside of the targeted cell which indicates a different metabolic state.

Disease Diagnostics

Major areas for disease diagnosis have been atherosclero-

sis, bone disorders and cancer of many forms.

As early as 1992, Michael Feld at MIT measured Raman spectra of a human artery as a means to extract information on the histochemistry of the **atherosclerotic disease process**^[13].

Then in 2006 the same group started making in vivo pathological assessments of atherosclerosis and vulnerable plaque^[14]. In 2012 the Jena group published an article describing measurements of atherosclerotic plaque in situ in a rabbit model using an endoscopic probe of 1mm outside diameter^[15]. Using band assignments in the literature for lipids (saturated, unsaturated, free fatty acids and esterified fatty acids, including triglycerides) and proteins, different areas of the aorta lumen were characterized in order to make predictive diagnoses of vulnerable plaque based on chemical composition. Subsequent to Raman measurements CARS (coherent anti-Stokes Raman scattering) images were recorded in normal aorta and aorta with plaque depositions. Because of the large CH vibrational frequency difference between protein and lipid (2930 vs. 2840 cm^{-1}), contrast in the CARS image was quite good.

Bone is a composite material of hydroxyapatite (a mineral) and collagen (a protein). In order to get the required mechanical properties, the bone is porous, and is undergoing constant remodeling by the osteoclasts and osteoblasts, forming osteons which are a tubular structures, parallel to the long axis of the bone, with concentric mineral lamellae. The mineral is substituted with non-stoichiometric carbonate on OH^- and PO_4^{3-} sites, and the crystallinity can vary; the relative mineral to protein ratio, carbonate substitution, and mineral crystallinity are all parameters that can be measured with Raman spectroscopy, and used to characterize the quality of the bone. In 1999 Professor Michael Morris at the University of Michigan published an early Raman study of bone which analyzed the spectroscopic characteristics of the components and applied multivariate methods to analyze line maps as a means to explore cortical bone chemistry^[16]. (Cortical bone is the dense, compact bone in the longitudinal center of the long bones.) Of significance in this publication is the finding of three different factors for the mineral phase with central Raman shifts for the phosphate band between 951 and 963 cm^{-1} . Morris and his group has continued his work on the study of bone, especially diseases of the bone^[17].

Subsequently Ozan Akkus and his collaborators used Raman microscopy to study the aging of human compact bone^[18]. In particular, they documented the composition of non-remodeled bone vs. newly formed bone in the

osteons from subjects between the ages of 17 and 73 years, with the goal of understanding fracture susceptibility. They found that the mineral content of primary bone (non-remodelled) increased over the first two decades of life, as did the size of the mineral crystals. However, the newer bone maintains an average larger protein to mineral ratio with smaller crystals, thus maintaining bone in a less brittle condition. They state that “slowing of remodeling may increase fracture susceptibility through an increase in proportion of highly mineralized tissue.”

For anyone interested in the possibilities of applying Raman microscopy to **cancer diagnosis**, a good place to start is a book chapter published in 2008^[19]. This chapter in fact concentrates on the diagnosis of bladder and esophageal disease, but the development of methods for these two cases should be seen as exemplary rather than unique to these tissue types. The current gold standard for cancer diagnosis is based on the conclusions of pathologists. However, it is well known that different pathologists examining the same specimens can reach different conclusions. So the protocol for developing Raman diagnosis involved collecting 150 biopsy samples, and submitting them to a group of pathologists for classification. Ultimately only 50 samples that represented consensus were used for Raman classification. Mean spectra representing normal tissue, Cardiac Barretts’ Fundic Barrett’s, Intestinal Barrett’s, low-grade dysplasia, high-grade dysplasia, adenocarcinoma squamous dysplasia and squamous cell carcinoma were shown. Linear Discriminant Analysis (LDA) was applied for classification; sensitivity and specificity for a 3 group model (normal squamous mucosa, Barret’s esophagus, Dysplasia and cancer) were 84-97% and 93-99% respectively. These values are as good as those of the pathologists but when organ removal is a consequence of diagnosis, one wants to minimize false positives. Likewise one would not want to miss cancerous tissue. Taking misclassification costs into account improved misdiagnoses.

Bladder specimens excised during cystoscopy yielded results similar to those cited above on esophageal cancer. In addition, it was found that the spectra from these samples could be used to stage the bladder disease, which is essentially how far the tumor has invaded the bladder wall. Ultimately, of course, one would like to provide in situ data in order to make immediate decisions. This can be done with a Raman endoscopic probe which, in this case, was a 6-around-1 fiber bundle with filtering to eliminate the Raman signal of the silica from the excitation fiber and the laser signal from the collection fibers; the probe used was an Enviva Raman probe from Visionex, Inc. Again using the LDA algorithm on ex vivo samples, benign samples (normal and cystitis) were differentiated

from transition cell carcinoma with 84% overall accuracy. This proof of principle supports the possibility of in vivo measurements in the operating room setting.

At the end of this chapter, written in 2008, these authors point out where caution needs to be practiced in applying Raman spectroscopy to cancer diagnosis. For a start, the spectra differences are quite subtle, so it could be helpful to decompose spectra into various components which will aid in the understanding of the disease process. The discrimination of benign from neoplastic tissue was done using multivariate methods. However, the question remained (at least at the time of the writing of this article) as to whether a model based on spectra collected on one instrument can be used for predictions of tissue state from spectra collected on a different instrument. Spectra can certainly be corrected for instrument response based on a broad band light source, but if spectra are collected on instruments providing different spectra dispersions, the relative intensities of bands of different widths will vary with the spectral dispersion, a phenomenon that has not been addressed as far as we know.

In 2015 Prof. Stone’s group wrote another review article on cancer diagnosis^[20]. In addition to discussing cancer diagnosis for a variety of tumor types (brain, breast, lung, skin, esophagus, prostate, colorectal, and bone) they also discussed biofluid diagnostic assays for cancer and other conditions. In this article they discuss the important issue of determining tumor margins. Collecting a full spectral image of an entire tumor with the patient in the operating room in order to determine the margin is prohibitive based on the time required, but algorithms have been developed to limit the number of sampling points by looking for spot to spot variations. This can be especially important in removing brain tissue where it is desirable to remove as much tumor as possible (in order to minimize the chance of recurrence) while not removing healthy tissue in order to avoid neurological deficit. They also review other measurement techniques such as CARS (coherent anti-Stokes Raman spectroscopy) and SRS (stimulated Raman spectroscopy), SERS (surface enhanced Raman spectroscopy) and TRS (transmission Raman spectroscopy).

Some of the studies cited in this article indicate that not only can Raman spectra with multivariate techniques be predictive, but sometimes the spectra do provide insights into molecular correlations with disease. For instance, in non-small cell lung cancer they found high porphyrin levels in healthy tissue but high DNA bands in tumor tissue. In another study that was cited autofluorescence measured with an endoscope identified suspicious regions which were then identified by Raman as pre-neoplastic

with 91% specificity.

In the discussion on skin cancer, the use of spectra to define tumor margins was explored to replace Mohs micrographic surgery which is costly. Discrimination between basal cell carcinoma and normal tissue was based on a classification model using the relative intensity of the bands assigned to nucleic acids and achieved $90\pm 9\%$ sensitivity and $85\pm 9\%$ specificity.

Professor Michael Feld and his group had been the first to explore utilizing Raman spectroscopy for tumor margin assessment^[21]. In particular, he identified one patient with an invisible cancer that required a second surgery after pathology confirmed the malignancy status. More recently it was shown that Raman spectroscopy can differentiate grey matter, white matter, necrosis, and pathologic glioblastoma^[22, 23]. In the first reference^[22] measurements were made with a 6×1 fiber probe coupled to a small portable spectrograph; the probe was brought into contact with a tumor that had been induced in a rat brain. The goal of this work was to develop a protocol for “the development of an in vivo Raman method for real-time intraoperative brain biopsy guidance.” In this and other studies, it was found that tumor development resulted in a reduction of lipid following demyelination of brain tissue. In the second reference^[23] bands assigned to protein and lipid were imaged and found to differentiate the phases mentioned, and these images correlated with adjacent H&E stained tissue sections.

Of particular interest is the potential of Raman spectroscopy to provide **diagnostic assays** directly in **biofluids**. Reference 20 that we have been discussing mentions six areas where this has potential: diabetes and glucose level monitoring, cancer diagnostics, asthma, inflammation, coagulant/anti-coagulant factors in blood and malaria. In 2008 Chan, et.al. demonstrated that spectra of individual leukemia cells could be acquired by taking advantage of laser trapping^[24]. Populations of both B and T cells from healthy individuals and leukemia patients were examined; DNA and protein markers were identified to have excellent discriminating power for cancer identification. Both PCA and LDA were used to verify the markers and classify the spectra.

But of highest importance is the combination of Raman spectroscopy of single cells in an optical trap in a microfluidic device^[25]. This will enable integration of Raman technology with standard fluid assays. Combined with enhancement mechanisms (such as SERS tags targeted to the malignant cell, or coherent processes such as SRS or CARS) there is the potential to identify a single metastatic cell in circulating fluid.

Raman Imaging

Histology has been developed in order to assess the physiological state of tissue. However it is an indirect means to assess the biochemical state of tissues, cells, and cellular organelles. Raman spectroscopy has repeatedly demonstrated its advantages and its effectiveness as a key technique for biology and biomedicine. Imaging and mapping are two widely used approaches, ex vivo and in vivo, for the investigation of various biomedical problems such as pathologies. Raman spectroscopy can be applied from the single cell to the tissue level, while providing structural and functional information in a non-destructive and label-free way.

In this section, we will point out the main advantages of Raman microscopy as a bio-imaging technique: it is label-free and non-invasive, it has high spatial resolution, and sophisticated multivariate data processing methods are available. We will show examples from the field of cell biology and tissue cancer medicine. There are many microscopic imaging techniques used to investigate the complexities of biological structures in cells and tissues, including optical microscopy, confocal laser scanning microscopy, scanning electron microscopy (SEM) and atomic force microscopy (AFM). These techniques provide highly detailed images, but they fail to characterize the chemistry and composition of the sample under examination. Spectroscopic techniques, however, can do just that, and in particular Raman micro-spectroscopy is fast becoming established for probing cellular biochemistry on the micrometer scale. The utilization of the confocal Raman microscope now allows this detailed chemical information to be acquired with microscopic resolution. Thus, the chemistry of cells and tissues can be quickly examined, and mapped images generated. These images contain full spectral content at each pixel, so that distribution of components can be visualized based upon their Raman spectrum. The arrival of instruments featuring inverted microscopes has taken the technique definitely into the area of the biologist.

Raman spectral imaging and mapping have become popular in the biomedical world and biochemistry first, because of the very low cross-section of water (many of the biological samples contain more than 75% water) and second, because of the high information content on the chemical and physical composition of the sample^[26]. The large amount of information from a Raman image or map requires appropriate tools for identifying and retrieving important parameters. This becomes possible when chemometrics (multivariate analysis MVA) is used for analysis^[27-30]. From a methodological point of view, it is important to distinguish between Raman mapping and

Raman imaging. Mapping is achieved by measuring a spectrum at a matrix of points on the sample having the coordinates $\{x, y\}$ which define the region of interest [28]; this method provides extensive chemical information from each point mapped and is confocal. Raman imaging allows quasi-simultaneous analysis of the entire surface of the sample or just a small portion at one specific wavelength. The final Raman image will be reconstructed from the measured spectra collected at small sampling steps over a selected wavenumber range [31]. When comparing the performance of Raman mapping with Raman imaging, it is usually noted that the acquisition time is much longer for mapping because a spectrum at each point in the image field is measured separately. However it should be noted that a legitimate time comparison would collect a global image at each wavelength which would diminish the time differences. However, as the technology advances, the time limitation of mapping is becoming less of an issue [32].

Some of the multivariate methods that have been applied to Raman maps include Principal Component Analysis

(PCA), Discriminant Component Analysis (DCA), Partial Least Squares (PLS), Multiple Least Squares (MLS) and Classical Least Squares (CLS). All these methods use the full spectral pattern rather than a single peak, extracting the variance in the spectra. Multivariate algorithms may be supervised if the user has a priori knowledge of the pure spectra, or unsupervised if s/he does not, and then allows the algorithm to “find” the factors that represent the variance.

An important barrier to capturing high quality Raman images is the presence of autofluorescence which tends to be a much more efficient process compared to Raman scattering. Many strategies have been developed to reduce the impact of the fluorescence; these include the use of deep UV [33] or NIR [34] laser excitation, shifted excitation Raman difference spectroscopy, stimulated Raman scattering (SRS), as well as many processing methods applied post acquisition. Another interesting strategy is to combine the use of Raman spectroscopy with intrinsic fluorescence spectroscopy and/or diffuse reflectance techniques.

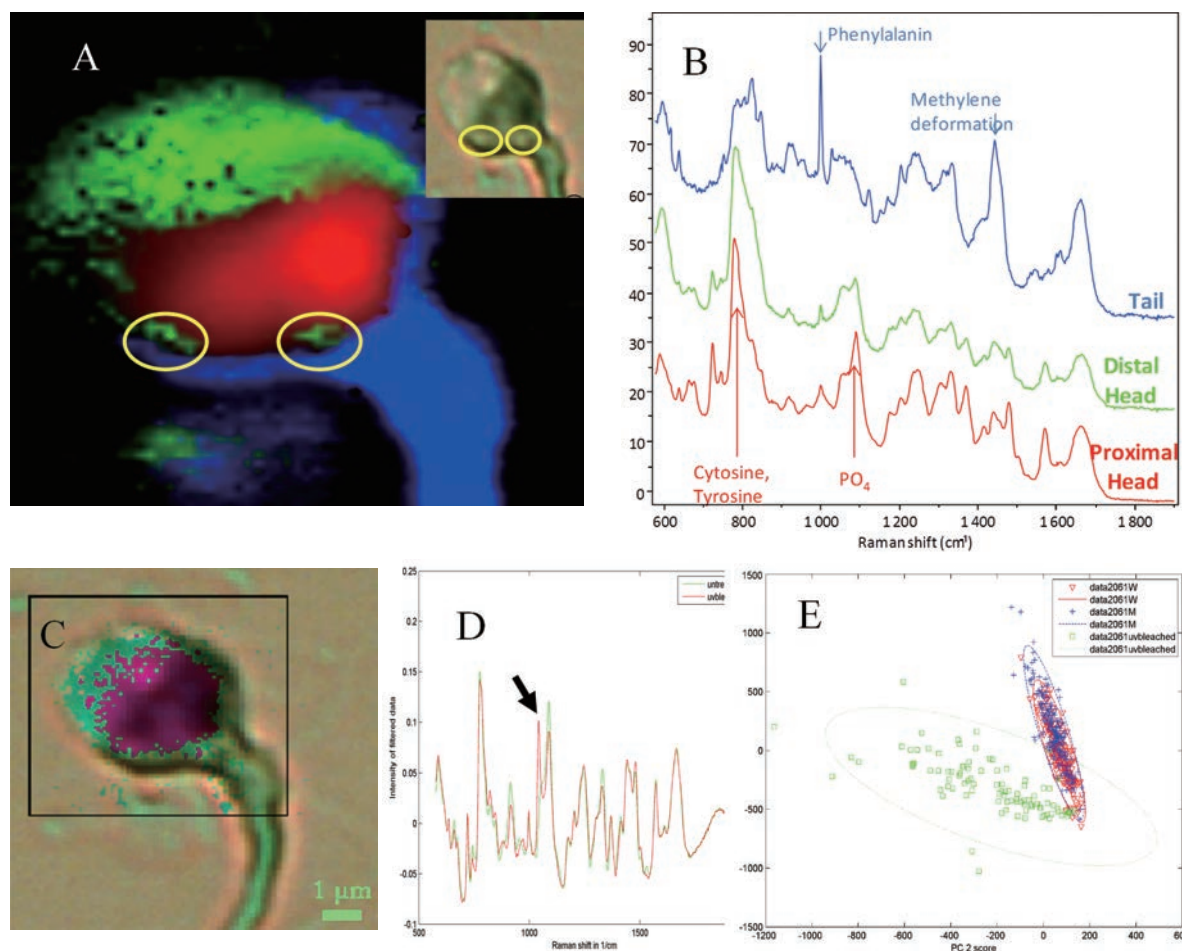


Figure 2 (A) (B) Raman map and the characteristic Raman spectra of native sperm (the video image of the sperm in the inset). Three distinct regions in the sperm are observed: proximal head, distal head and tail, as well as small details as vacuoles. (C) Overlay of human sperm showing the locations of spectra found to be indicative of intact (purple) and damaged (aqua) DNA. (D) Averaged spectra showing the distinct shift at 1042 cm^{-1} (arrow) in spectra of irradiated (red) compared with that of the untreated human sperm (green). (E) Scores cluster plot derived from PCA: native sperm (red and blue, for two observers) and UVB-irradiated sperm (green).

Single cell analysis

As an example, Prof. Con Mallidis and co-workers from Center for Reproductive Medicine and Andrology, University Clinic in Munster studied the sperm cell; more precisely, they were interested in the sperm DNA and the influence of UV irradiation on these entities^[35]. The aim of the study was to analyze with Raman imaging technique the sperm nDNA status in normal and infertile cells (affected by UV radiation) and to compare the results with

established nDNA tests.

Air-dried human sperm smeared on a suprasil microscope slide were mapped with high spatial resolution. By assigning a specific color to each of the chemical signatures, different regions of the sperm cell can be identified (Figure 2A, 2B). The image was created using the Classical Least Square method. Three distinct chemical profiles were identified corresponding to the proximal

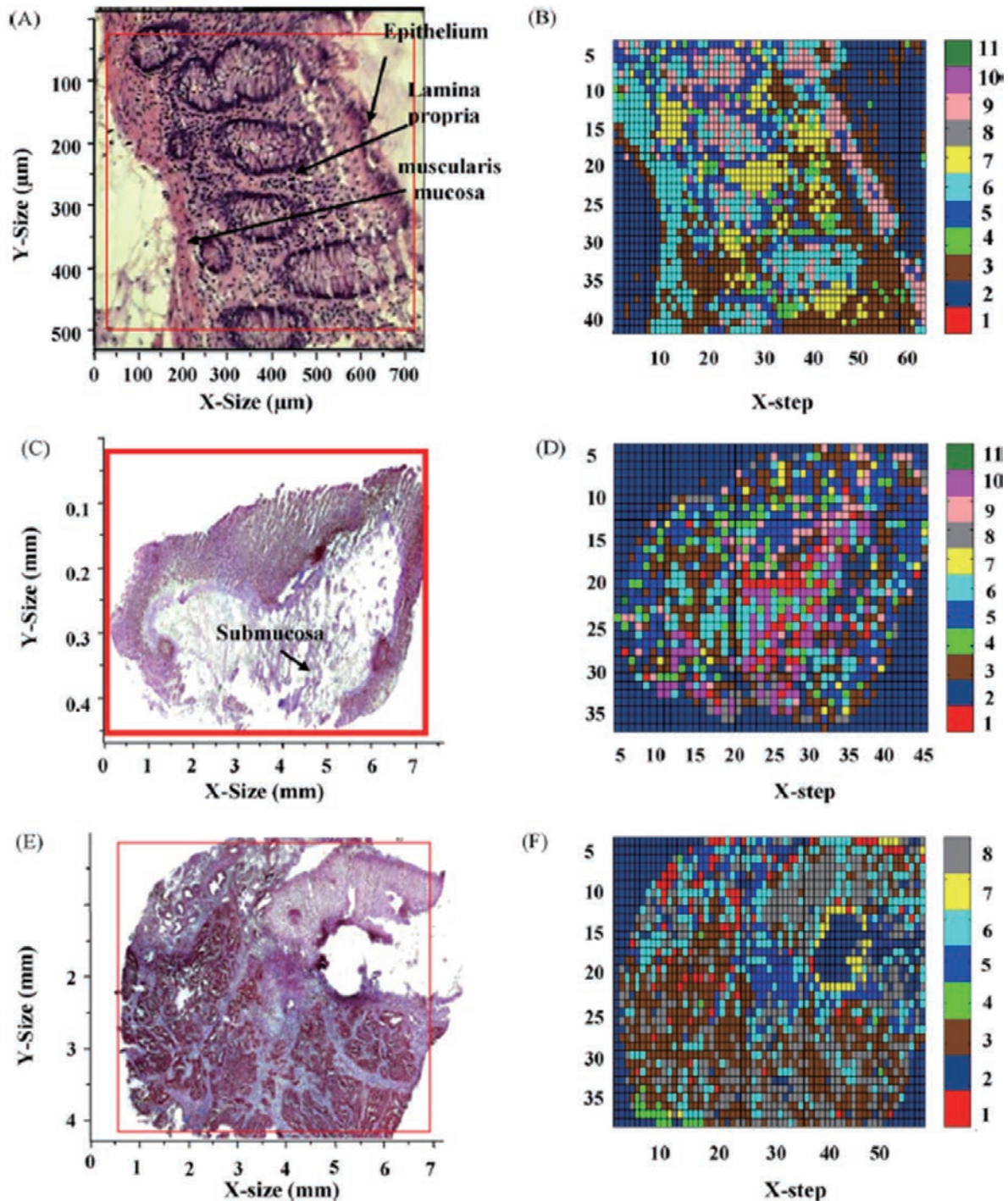


Figure 3 Histopathologic HE-stained section of normal (A and C) and of adenocarcinomatous (E) colonic tissues; B, D and F display pseudo-color Raman maps based on 11-means cluster (B and D) and 8-means cluster (F) on the same areas of the adjacent unstained section. Each cluster (consisting of similar spectra) was assigned to one color and corresponded to the extracted model.

head (containing DNA), the distal head (Acrosome) and the tail of the sperm (Flagellum). The high spatial resolution of the Raman map allows the detection of small irregularities in the sperm head such as vacuoles (yellow circles).

The native sperm cells were closely compared with the sperm cells after UV irradiation which was expected to produce changes in nDNA. Standard PCA analysis was performed on three data sets: native sperm/observer W, native sperm/observer M and damaged sperm. The results show that no difference is observed between the different observers' measurements on native sperm however they were clearly distinguishable from those obtained after UVB treatment (Figure 2E). Spectrally, the main difference between native and UV-treated samples concerns the DNA PO_4^{3-} vibrational band (Figure 2B). This difference indicates the dimerization of nucleotide bases caused by UVB. The spectral shift can be used to visualize the distribution of damaged and undamaged DNA within the single cell (Figure 2C).

Ex-vivo tissue examination

In cancer research and patient care, traditional methods of diagnosis are based on histopathological staining and a (trained) human eye (the pathologist), but such methods can often only distinguish between very different 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 microspectroscopy. 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 tumor's

development stage and its malignancy.

Professor Manfait and co-workers at the University of Reims, France, have been harnessing the power of Raman micro-spectroscopy for the life sciences, and recent work has focused on medical applications of Raman spectroscopy to differentiate between normal and adenocarcinomatous human colonic tissues^[36]. These tumors are particularly aggressive, spreading quickly and often evading traditional surgical techniques for removal.

Figure 3 illustrates results from mapped Raman imaging of an unstained normal and adenocarcinomatous colonic tissue section.

The spectroscopic data were correlated with the gold standard histopathological technique. Multivariate statistical analysis (principal component analysis (PCA), clustering analysis (CA), and the multiple least squares algorithm (MLS)) were used to investigate the spatial distribution of molecular changes in individual colon constituents (proteins, lipids, collagen, nucleic acid, and mucus). The images displayed in Figure 4 show the distribution of protein, collagen, lipids and mucus in normal and adenocarcinoma tissues of these colon specimens.

The authors observed that, for the healthy tissue, the proteins are present in the mucosa, while the lipids and collagen are localized in the submucosa, and the mucus surrounds the tissue. Also, one can note that the DNA is highly distributed in the tumor part of the tissues separated with stroma, and mucus around the tissue. The multivariate method used (MLS) confirms the absence of the lipids in adenocarcinoma tissue and the presence of the

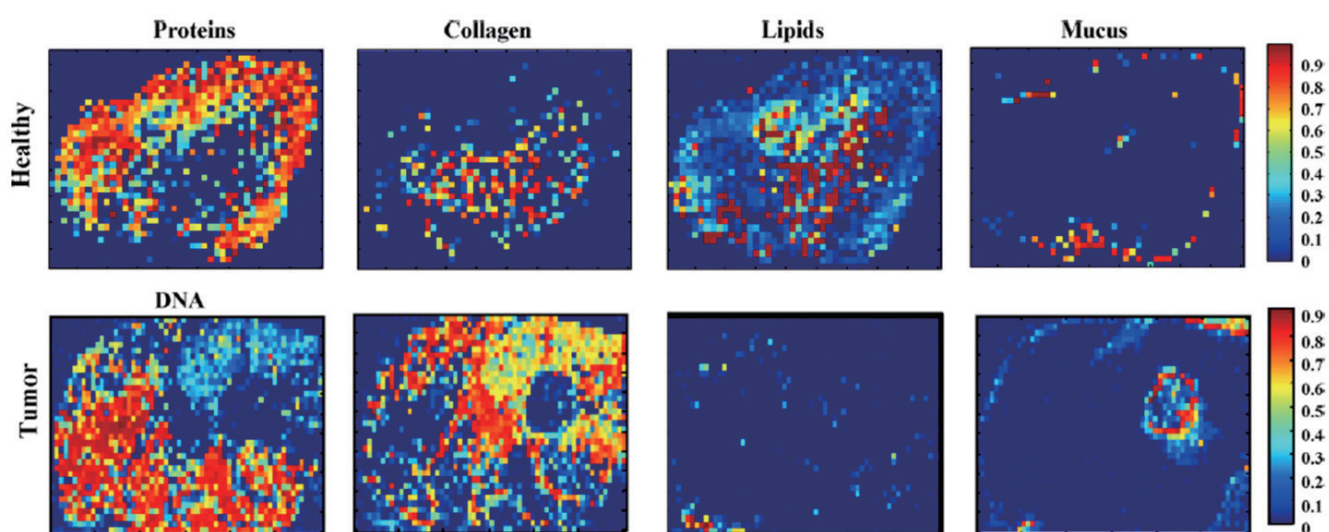


Figure 4 Biochemical distribution of the individual constituent (proteins, collagen, lipids, DNA, and mucus) between normal, adenocarcinoma colonic tissues produced by applying the MLS algorithm. To determine the molecular composition of tissue composition, the relative scattering intensities of the various components were normalized to add up to 100%. The spatial and biochemical information obtained from these maps can be used to identify which biochemical markers could discriminate between normal and cancer tissues

collagen around the stroma.

The take home message from these imaging studies indicate that Raman micro-spectroscopy can identify molecular changes that occur during the early stage of malignancy, at a time when histological/morphological evidence is lacking.

Concluding Remarks

The application of Raman spectroscopy to identification of bacteria species and strains, to disease diagnosis, and to molecular mapping has been surveyed. We have attempted to credit people in the field, especially those who have explored certain areas for the first time. But the field is growing rapidly, and an examination of some of the references cited here indicates that there have been thousands of publications that have appeared in the last 10 years. Thus this article should be viewed as an introduction to the field, in order to understand what the potential is for Raman spectroscopy.



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