Fall 2008

This newsletter is produced by HORIBA Jobin Yvon's Molecular & Microanalysis Team, to provide our customers, colleagues & friends with up-to-date information in the fields of Raman, fluorescence, SPRi & XRF Instrumentation and Applications.

What's inside:

Combined analysis of biliary calculi by Raman and XRF Microscopy

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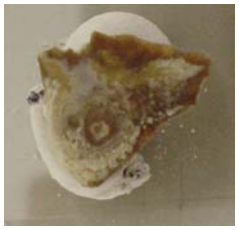


Fig. 1: Video Image

The recurrence of megalgia by calculus is terrible for the patient who suffers from lithiogenic disorder. To prevent the recurrence of the calculi production, it is necessary to to monitor food intake. For this purpose, we must know the components and the interaction of these components for the process of crystalline nucleation, aggregation and growth of calculi. Infrared spectroscopy has been often used for the components-analysis of calculi. Information from this technique, however, is limited to the averaged components-ratio.

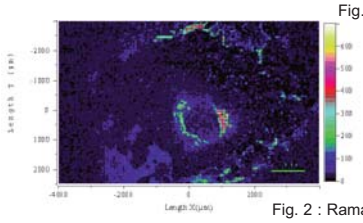


Fig. 2 : Raman Image

Mapping techniques using near-IR-Raman microscopy (HORIBA-Jobin-Yvon LabRAM HR-800) and X-ray Fluorescence microscopy (HORIBA XGT-5000) were applied for morphological observation of components included in the 8 mm x 6 mm area around nucleus of biliary calculi. We first reported results about components distribution and morphological information from both Raman and X-ray fluorescence images as shown in Fig1. They provide important information to reveal the process of crystalline nucleation, aggregation, and growth of calculi. In the case of the calculus in Fig. 1, apatite accumulated in the nucleus conjugated with Zinc and generated small ring-shape distribution. Ca-based oxalate was distributed in the surrounding area of the nucleus. The second ring distributed in the surrounding of the nucleus included mainly apatite. These results suggested that Zinc played an important role for the generation of the nucleus.

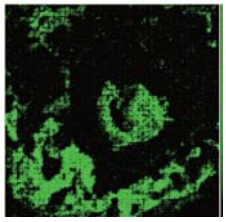


Fig. 3 : XRF Image

Elemental Mapping of trace elements in tree bark pockets using micro-XRF

Dr Simon FitzGerald, Principal Scientist, HORIBA Jobin Yvon Ltd
Prof. Cameron McLeod, Centre for Analytical Sciences, Department of Chemistry, University of Sheffield

There continues to be growing concerning about chemical pollution and its adverse effects on the environment and human health. Part of this work focuses on the monitoring of historical pollution levels since this allows long term trends to be studied.

One interesting approach for such monitoring is the measurement of trace elements in bark pockets in trees.[1] Bark on trees continually accumulates pollutants from the atmosphere, and as a result outer bark cannot be used for historical monitoring. However, bark pockets occur when some of the outer bark folds in on itself and then becomes sealed within the tree. Since the bark is now sealed, it contains pollutants which have been accumulated only up to the point when the pocket was formed. The pockets are formed between the annual growth rings of the tree, which allows them to be very accurately dated. Thus bark pockets offer a unique opportunity for historical environmental monitoring.

The XGT-7000 x-ray fluorescence (XRF) microscope has been used to study the distribution of elements within a bark pocket. Unlike other techniques such as laser ablation or ICP which have previously been used for these types of analyses, XRF is completely non-destructive and offers high spatial resolution for both single point quantitative analyses and element imaging. It allows the bark pockets to be retained as a part of the historical record and to be analysed using other complementary techniques too.

By scanning the 25mm x 25mm sample beneath the X-ray microbeam the elemental structure of the bark pocket can be probed. As Figure 1 illustrates, the bark pocket can be readily distinguished, elementally, from the bulk tree tissue by the high concentrations of Calcium and Potassium. A region of high Iron and Zinc concentration is observed at the centre of the pocket, which is surmised to be due to accumulation of airborne pollutants.

Trace levels of Lead were also observed, although signal intensity is relatively weak – Figure 2 shows the image of Lead distribution, with an overlay of the peak intensity across the bark pocket . Spectra acquired from the bulk wood tissue and from the interface region clearly show the presence of many trace elements, not least the distinct Lead Lα and Lβ peaks.

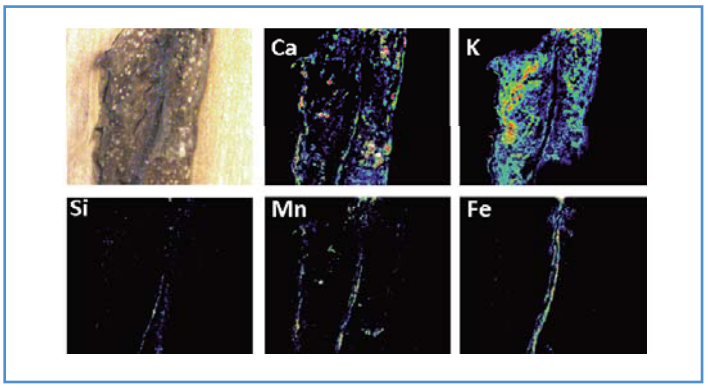


Figure 1: optical and element distribution images acquired using a 100 µm x-ray beam.

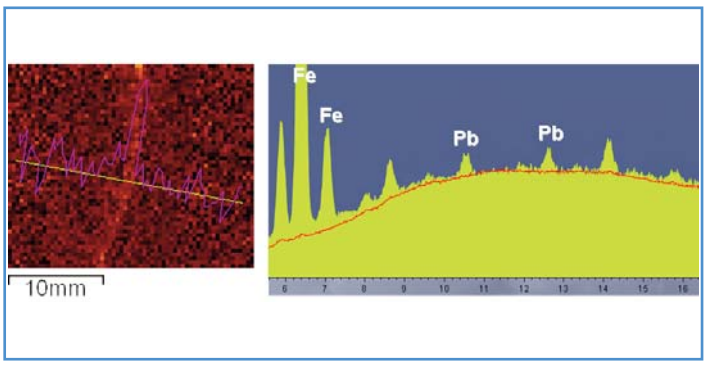


Figure 2: low resolution Lead element image (400 µm pixels) with line profile across the bark pocket . The clear presence of lead in the centre of the bark pocket is seen in the accompanying spectra (bulk wood tissue [red] and bark pocket [yellow].)

Macro and Micro Localization of β-carotene

Dr. G. Le Bourdon, S. Morel, Horiba Jobin Yvon SAS

When buying salmon, the colour of the flesh usually does mat
presence of beta-carotene pigments and therefore understanding and c
is of great interest for the salmon industry.
The unique Raman macro-mapping capability of the DuoScanTM option
ple consisting of a very thin slice of carrot, starting with a macro-scale s
Fig.1 shows an extended video image of the carrot thin slice obtained b
the objective being used. The blue line represents the area mapped wit
the entire surface, thereby avoiding blind regions. The average spectrur
carotene pigment and to obtain somewhat a binary image where the gre
8 mn.
In a second step, micro-Raman mapping was undertaken on the bright a
tribution, but at the micron-scale this time. The resulting image shows a
of about 5 x 5 µm.

Sample courtesy : Dr Nils Afseth, Matforsk, Norway

Confocal Raman Microspectroscopy as a spatially resolved probe for Microfluidics

JB. Salmon, F. Sarrazin, G. Cristobal and B. Pavageau LOF, UMR 5228-Rhodia- CNRS-Universite Bordeaux 1.

L. Servant, D. Talaga and JL. Brunel Institut des Sciences Moléculaires, UMR 5255-Universite Bordeaux 1.

Microfluidics encompasses the science and technology of handling fluids using channels with dimensions in the micrometer range. This field faces presently a tremendous interest, especially for the development of lab-on-a-chip systems providing the integration of complex chemical and biological assays on a microfabricated chip. Beyond the obvious interest of miniaturization, these devices allow integration and economy of scale, and offer unique opportunities in the manipulation of fluids (mixing, transport…) that can be created through the design of clever channel architecture. A major issue of significance lies in the probes that can be used to characterize the fluids at these scales. Raman Confocal microspectroscopy (CRM) appears, in principle, ideally suited to do the job: its intrinsic selectivity does not require the use of specific label, its spatial resolution is typically in the micrometer range and, last but not least, its low sensitivity is no longer a hindrance, for most systems operate under stationary conditions. In a joint research effort, researchers from ISM and LOF have investigated the coupling of CRM with microfluidic devices. Two-chips devices were studied: in one, two incoming streams (H_2O and D_2O) met in a single larger channel where they flow adjacently and interdiffuse to react and give HOD. Straightforward spectral analysis provides a way to give the spatial distribution of H_2O, D_2O and HOD species.

In another chip design, the idea is to engineer spontaneously nanoliter droplets of uniform size from microchannel jonctions, where two immiscible fluids streams merge, resulting in the formation of tiny droplets acting as chemical reactors. Mixing and transport issues were addressed using CRM: beyond the case of the model reaction of water deuteration, the Raman data collected on chips under stationary conditions should be able to provide insights in reaction-diffusion schemes of chemical reactions. Interestingly, such results suggest further applications in the investigation of fluid rheology.

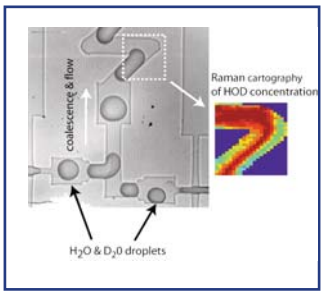


Figure. 1. In-Situ Raman probing of travelling droplets' composition

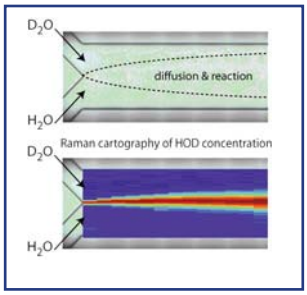


Figure. 2. In-Situ Raman probing of coflow streams

Droplet control for microfluidics , M. Joanicot, and A. Ajdari , Science 309, 5736 (2005).

In situ Raman imaging of interdiffusion in a microchannel, J.-B. Salmon, A. Ajdari, P. Tabeling, L. Servant, D. Talaga, and M. Joanicot Appl. Phys. Lett. 86, 094106 (2005).

On-line laser Raman spectroscopic probing of droplets engineered in microfluidic devices . G. Cristobal, L. Arbouet, F. Sarrazin, D. Talaga, J.-L. Bruneel, M.Joanicot, and L. Servant , Lab Chip 6, 1140 (2006). Chemical Reaction Imaging within Microfluidic Devices Using Confocal Raman Spectroscopy: The Case of Water and Deuterium Oxide as a Model System , F. Sarrazin, J.-B. Salmon, D. Talaga, and L. Servant Anal. Chem. 80, 1689 (2008)

e pigment in Salmon using Raman DuoScan™ Imaging

ters at least as much as the ultimate taste of it, if not more. The intrinsic color is due to the controlling the distribution and concentration of pigments in function of the feeding process

allows investigating the spatial distribution of the β-carotene pigment within a model sam-urvey followed by a more detailed analysis at the micron-scale.

by successive recording and merging several images acquired through the field of view of h the DuoScan device using a preset square macro-spot of 20 x 20 µm allowing covering m collected from each macro-pixel allows to emphasize the presence or absence of the β-en spots denote the pigmented regions. This 180 x 200 µm area was scanned in less than

area on the bottom left side of the macroRaman map to get an insight into the pigment dis-location of the pigment mainly on the top right corner of the covered surface, over an area

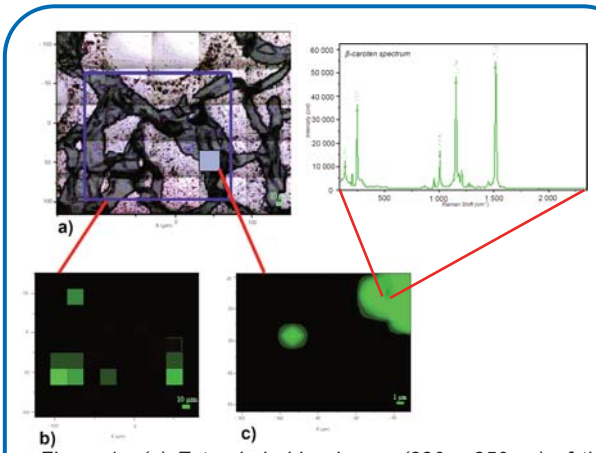


Figure.1 : (a) Extended video image (220 x 250µm) of the carrot slice with the (b) macro-mapped and (c)micro-mapped areas respectively indicated with the blue line and light blue rectangle.

TemPro: TCSPC Lifetime Fluorescence Everyone Can Afford

By Stephen Cohen, Ph.D

Determination of fluorescence lifetimes often places a strain on research budgets. Finally, with the new filter-based TemPro (Fig. 1), reliable fluorescence lifetimes are now within everyone's price-range. The basic TemPro uses a time-correlated single-photon counting (TCSPC) acquisition mode for fast kinetics and detection of lifetimes from <100 ps to seconds. The excitation source is our pulsed solid-state NanoLED for shorter lifetimes, and SpectraLED for longer ones, available from the UV through the near-IR. Fully modular construction eases upgrades and customization. The unique combination of photon-counting detection and optional vertically dispersive optics achieves the ultimate in sensitivity.

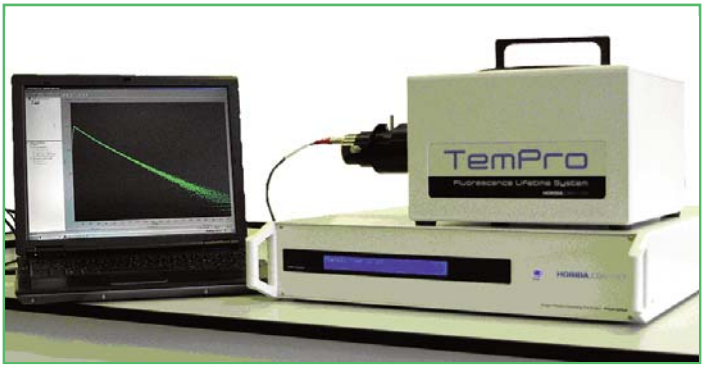


Figure. 1. HORIBA Jobin Yvon's new affordable TemPro TCSPC lifetime spectrofluorometer

The TemPro uses our TBX-04 detector, with wavelength range of 185–650 nm. Upgrades include a cuvette-holder with digital temperature sensor and software-controlled magnetic stirrer; automatic and manual polarizers; front-face sample-holder; and extended detector response to 850 nm. For the ambitious researcher, the TemPro even can be upgraded to our premier FluoroCube system. An example of data from the TemPro is Fig. 2, a TCSPC lifetime decay of europium decatungstate (a potential new biological label) excited at its $^7F_0 \rightarrow {}^5L_6$ transition by a 395 nm pulsed SpectraLED solid-state source. Emission was selected with a 550-nm long-pass filter. Time per channel was ~2.67 µs. An excellent bi-exponential fit to the data gives a short fluorescence lifetime of 246 µs and a long lifetime of 2.20 ms.

The TemPro is a valuable addition to any laboratory, and is easy to use with our dedicated DataStation software forWindows®. If you are seeking an economical, true picosecond-lifetime spectrofluorometer with all the know-how and service that HORIBA Jobin Yvon offers, and that saves space on your laboratory bench, the TemPro is for you.

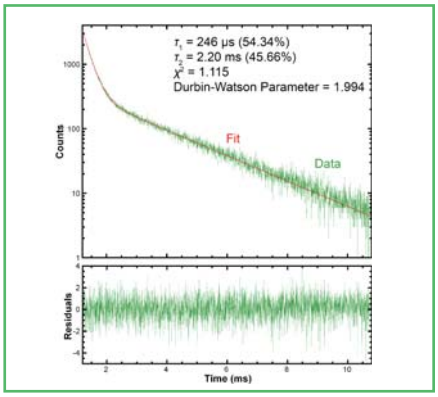
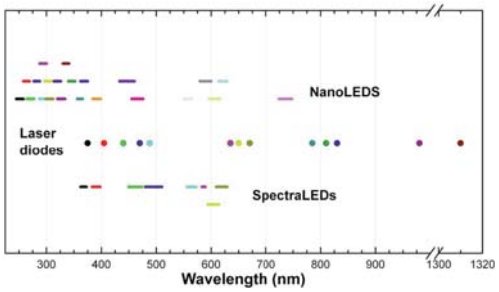


Figure 2. Decay (top) and residuals (bottom) of europium decatungstate excited with a 395 nm SpectraLED. A 550-nm long-pass filter selected for emission. In the upper plot, green points are the data, while the red line is the bi-exponential fit. In the lower plot, green points are the difference between data and fit. Calculated fluorescence lifetimes are 246 µs and 2.20 ms, contributing nearly equally to the luminescence.

APPLICATIONS

- Fret (Förster Resonance Energy Transfer)
- Stern-Volmer Quenching
- Efficiency of photosynthesis
- Deconvolution of mixing processes
- Fluorescence anisotropy
- Protein denaturation
- Coral fluorescent lifetimes



Our versatile pulsed solid-state excitation sources: NanoLED and SpectraLED

Identification of biomolecules captured by Surface Plasmon Resonance using MALDI mass spectrometry

S. Bellon[1,2], W. Buchmann[2], F. Gonnet[2], N. Jarroux[3], M. Anger-Leroy[1] and R. Daniel[2]

1 GENOPTICS Bio Interactions, 2 Université d'Evry-Val-d'Essonne, LAMBE, 3 Université d'Evry-Val-d'Essonne, LMPI

SPR allows thermodynamic analysis of biomolecular interactions. Mass spectrometry is a powerful tool for structural characterization and identification. Therefore, the combination of SPR and MS into one concerted procedure is of a great interest for functional and structural analysis within the fields of proteomics, drug-discovery, diagnostic, and bio-security. Most of the recent developments aiming to the SPR-MS coupling are based on the elution and micro-recovery of the captured bio-molecules, adding a time consuming step and leading to material loss. To overcome these limitations, we have implemented an on-chip MALDI analysis in which affinity captured bio-molecules are directly detected from the SPR-sensor surface.

For that purpose, we have used an SPR-biochip in a micro-array format. The biochip was functionalized by a self-assembled monolayer of polyethylene oxide carrying a NHS group reacting with primary amines. The validation of the system was performed by measuring antigens-antibodies interactions by SPR, and by MALDI detection of the retained antigens directly from the biochip. Two types of antibodies Anti-β-lactoglobulin and Anti-ovalbumin, were arrayed by coupling to NHS-functionalized biochip (SPRi-slide™). The SPR experiments were performed using a MS compatible running buffer. A mixture of proteins (β-lactoglobulin and ovalbumin) diluted in the running buffer was injected and analyte capture was followed in real time. (Fig 1)

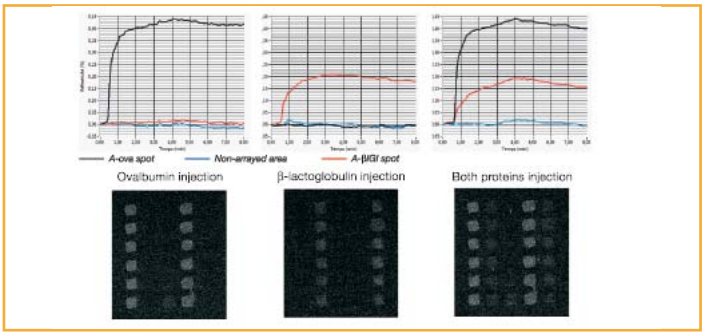


Figure 1: Antibody / protein interaction (— A-ova spot, — A-βλGl spot, — Non-arrayed area)

After SPR experiments, the biochip was removed and inserted directly in an appropriate MALDI-MS plate holder. Finally, the MALDI matrix was directly dropped onto the biochip and mass spectra were obtained from distinct spots. The representative mass spectra obtained from the protein-bound antibody array showed multiply-charged protein ions corresponding to the specific antigen, without any trace of non-specific binding (Fig 2).

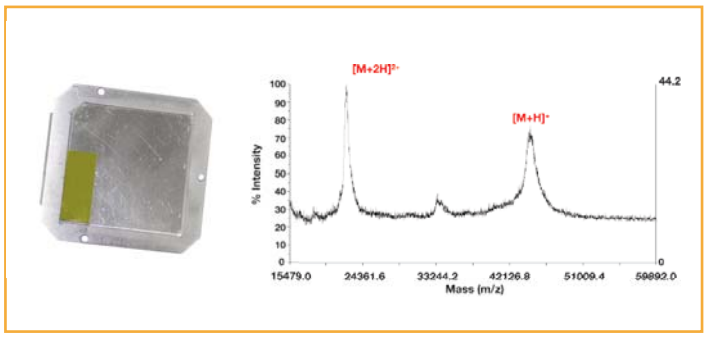


Figure 2: Mass analysis of retained ligand on anti-ovalbumin spot (SPRi slide sensor surface inserted into a MALDI plate)

Furthermore an additional on-chip digestion of distinct spots allowed the MS protein identification of the specific bound analyte captured by SPR (Fig 3).

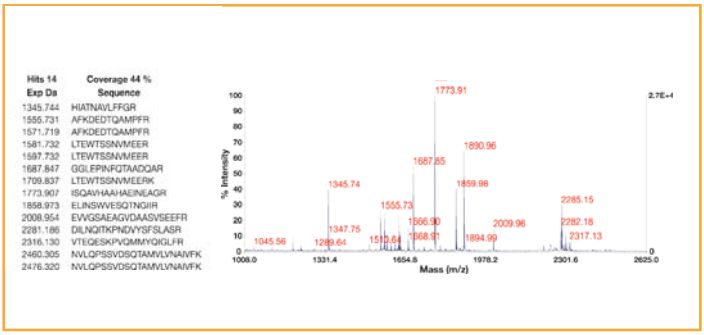


Figure 3: Mass analysis of retained ligand on anti-ovalbumin spot (MASS analysis following on chip digestion of retained ligand on anti-ovalbumin spot: ovalbumin peptides identification)

The SPRi/MALDI-MS combination on an array platform is a powerful tool for affinity separation and subsequent identification of compounds issued from complex solutions. The biochip developed in this work, the SPRi-slide™, was easily transferable from SPR apparatus to the MALDI-MS plate, and MS analysis was directly carried out on micro-array biochip. High-throughput multiplexed analysis with spatial resolution is permitted with this array platform.

INNOVATIVE ASPECT - Ligand fishing by SPR and subsequent MALDI-MS identification of the retained ligands have been carried out from the same surface.

HORIBA Jobin Yvon present at FACSS

As mentioned in previous issues, the FACSS conference in Reno, NV, was again a major event for the Spectroscopy community and gathered many scientists from all over the USA and foreign countries. This year again, many sessions were dedicated to new and emerging application fields for Raman, Infrared, and mass spectroscopies…etc.

HORIBA was strongly present with our largest booth we have ever displayed at FACSS. The Molecular and Microanalysis Division exhibited the new XploRA our low cost Raman microscope, the LabRAM ARAMIS, the XGT 7000 EDXRF microscope, the FluoroMax (r) - 4 and the MF2 multifrequency lifetime fluorimeter. We also contributed six presentations to the scientific program (see below).

FACSS is a very important event for our world-leading Raman team and again the program was dominated by Raman spectroscopy, with over 125 papers. Transmission Raman, SERS and TERS were major topics with many interesting presentations.

On Tuesday afternoon we had free hot dogs and beer in our booth to thank our customers for their continued support . Tthis proved to be a great success, with many people excited by the new XploRA and MF2 instruments.

FORTHCOMING EXHIBITIONS

10-13 January
ARAB LAB
Dubai

20-21 january
ISRANALYTICA 2009
Israel

February 2009
ICON 2009
Melbourne, Australia

February 2009
IITALIAN RAMAN CONGRESS
Milan, Italy

10-12 February 2009
FARMA&MAG
Zaragoza, Spain

8-13 March 2009
PITTCON 2009
Chicago, Illinois, USA

April 2009
OPERANDO III
Rostock-Warnemünde, Germany

To find out about other conferences and exhibitions at which HORIBA Jobin Yvon shall be present consult our website: www.jobinyvon.com

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