

Autumn 2007

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 and XRF Instrumentation and Applications.

See us at:

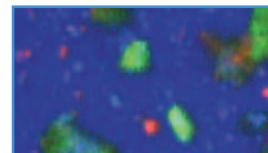
MRS Fall

**26-30 November 2007,
Boston, MA, USA**

What's inside

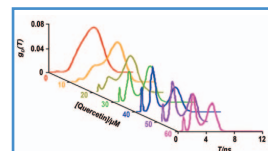
Page 2: Raman imaging at the speed of light

New developments in Raman microscopy make Raman imaging faster and more versatile than ever.



Page 3: Detecting Conformational Rotamers with TCSPC

Our TCSPC lifetime spectrofluorometer is ideal for detecting conformational changes in proteins when complexed with smaller molecules such as flavonoids.



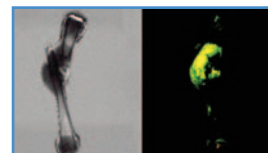
Page 4: SPRI-Lab+™ and SPRI-Plex™: Label-free and real-time biomolecule interaction detection

The SPRI is the ideal solution for the development of label-free, real-time and multiplexed bioassays for the detection of biomolecule interaction.



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X-ray analysis of a frog bone provides insight into the original fracture position and its healing mechanisms.



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Discover the award-winning MF².



Page 6: Our forthcoming conferences and shows

The XGT-7000 comes to EuroAnalysis

HORIBA Jobin Yvon introduced the latest X-ray Fluorescence micro-analyser to Europe at EuroAnalysis 2007, the XGT-7000. EuroAnalysis, held in Antwerp, Belgium in September is a central forum for discussion and presentation of up-to-date activities in Europe regarding Analytical Chemistry.

The XGT-7000 is the most recent member of the XGT series. Mr Yoshihiro Yokota from HORIBA (Japan) presented the XGT-7000 and discussed its new features. This innovative new system offers:

- Non-destructive EDXRF elemental micro-analysis
- High spatial resolution (10µm)
- Qualitative and quantitative analysis
- Unique dual vacuum modes
- Full hyper spectral XRF imaging



The HORIBA Jobin Yvon booth at EuroAnalysis 2007.

Raman imaging at the speed of light

Arnaud Zoubir & Alexandra Rapaport,
HORIBA Jobin Yvon S.A.S. Villeneuve d'Ascq, France

Raman imaging has long been used to produce chemical images at the sub-micron scale, providing crucial information to experimentalists working in the fields of pharmaceutical research, semiconductors, geology, etc.

Although this technique is non destructive and requires no sample preparation, it is sometimes seen as rather slow in comparison to other imaging techniques such as NIR or fluorescence microscopy. HORIBA Jobin Yvon was the first to introduce the patented LineScan technique where the laser beam is raster-scanned along a line. This produces faster acquisition times as all spectra along the line are taken simultaneously.

Now, we have taken things much further, pushing the limits of our systems to obtain high quality Raman images with unprecedented speeds of acquisition.

Introducing Scanning With Incredibly Fast Times: SWIFT™ Mapping

With point-by-point Raman mapping, a lot of the acquisition time is wasted in communication between the hardware and the software.

Recent advances in detection and hardware communication have enabled us to reach measurement times as low as 7 ms/point, opening the door to quasi-instantaneous chemical imaging. This revolution has a name: SWIFT™ mapping. Fig. 1 below, shows a Raman map of Pharmaceutical crystals obtained in 80 sec, less than the time needed to read this article!

SWIFT™ mapping can be used in combination with EMCCDs for optimal results, but also with standard CCDs, allowing possible upgrades on existing systems. Do you have a need for speed? Ask us how you can break the speed limit of Raman imaging.

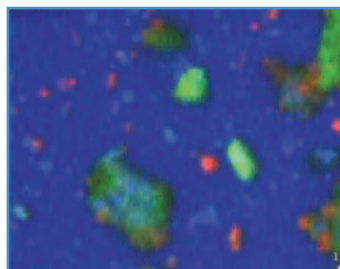


Figure 1 : Raman image of Pharmaceutical crystals obtained in SWIFT mode (47 x 40 μm , 120 x 50 data points). Total acquisition time: 80 sec

Get the full picture of your sample: Introducing DuoScan™

HORIBA Jobin Yvon has launched a new imaging mode that will allow you to shed new light on your sample. The revolutionary DuoScan™ (patent pending) offers the best of both the micro and the macro world, as it generates Raman maps across both μm -scale and cm-scale areas with FULL coverage of your sample (Fig. 2).

When looking at large sample surfaces, whether it is to measure component distribution or to search for contaminants, it often comes down to finding a needle in a haystack. The DuoScan allows you to vary the spot size from 1 μm to 300 μm to match your image pixel size, ensuring that you don't miss a spot even on very large sample areas (Fig. 2). The images produced are fully confocal, to better reject fluorescence or signals coming from beneath the analyzed surface.

Moreover, this technology can be used with excitation from the deep UV to the NIR. So, whatever your application, your sample size or laser requirements, we've got your sample covered!

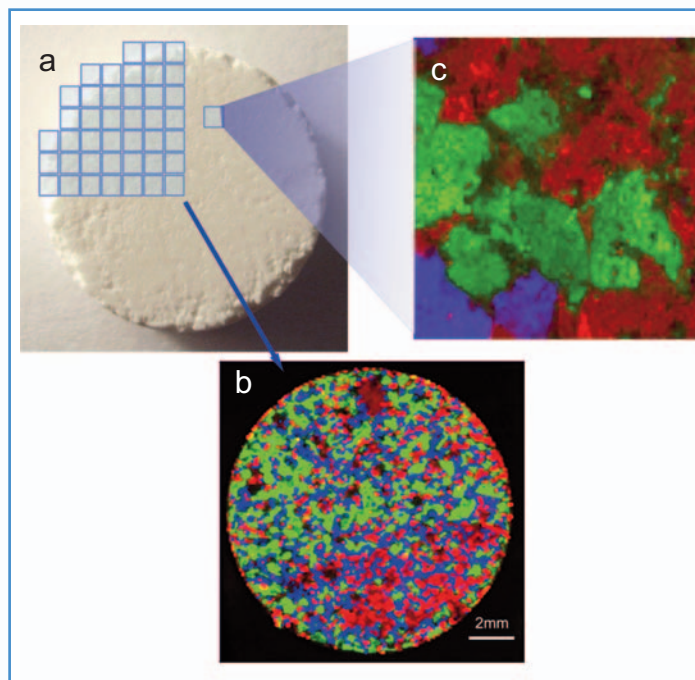


Figure 2:
(a) White light image of a Pharmaceutical tablet
(b) DuoScan™ large area mapping on a full tablet. 100% of this 12.5 mm diameter tablet was imaged in about 10 minutes
(c) DuoScan™ point-by-point mapping on a region of interest for finer analysis, pixel size: 1 μm

Detecting Conformational Rotamers with TCSPC

David Birch¹ & Stephen M. Cohen², ¹University of Strathclyde in Scotland, ²HORIBA Jobin Yvon Inc., Edison, NJ, USA

Among possible fluorescence biosensors for medical and biochemical monitoring and imaging are flavonoids, antioxidants in many botanical products such as tea, chocolate, and red wine. Flavonoids bind to nucleic acids and proteins. Many flavonoids, like proteins, are fluorescent, thus allowing Förster resonance energy-transfer (FRET) between the two types of compounds when they bind together. Dr. Olaf Rolinski and Professor David Birch, at the University of Strathclyde in Scotland, have investigated complexation of human serum albumin (HSA) with the flavonoid quercetin (Q), using time-domain fluorescence spectroscopy¹. Aqueous 0.01-M phosphate-buffered HSA (30 μ M) solutions to pH 7.4 were mixed with Q dihydrate (0–60 μ M). Time-correlated single-photon counting (TCSPC) spectroscopy was performed using our 5000U fluorescence lifetime system. The excitation source was our NanoLED ($\lambda = 295$ nm, pulsewidth ~ 0.6 ns), run at 1 MHz, with a time per channel = 7.06 ps. This wavelength corresponds to the absorption of the sole occurrence of tryptophan in HSA (position 214). Deconvolution of the fluorescence decay from the excitation pulse was achieved with our exclusive DAS6 software, giving best fits via minimizing the goodness of fit, χ^2 .

Fluorescence was recorded at 340 nm, the emission peak from tryptophan. Data were fit initially to a tri-exponential function, consistent with a widely accepted model of three rotamers, i.e., three conformations of tryptophan in HSA (Table 1).

Lifetime component	Lifetime (ns)	Contribution (%)
1	0.793	2.05
2	4.089	44.59
3	7.145	53.35

Table 1. Lifetimes and their contributions in pure HSA, using a tri-exponential model.

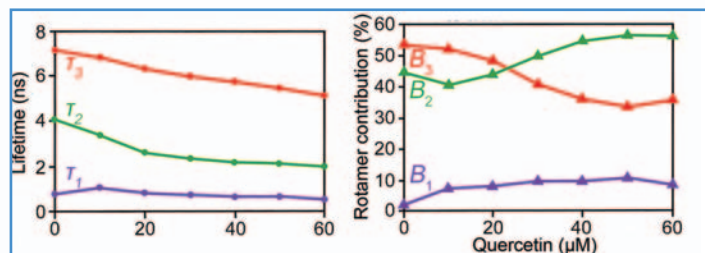


Figure 1 : Results from a tri-exponential model. Left: Change in each HSA lifetime with [Q]; right: change in relative contribution for each corresponding rotamer.

As the concentration of Q was increased, the lifetime components and their relative contributions changed (Fig. 1).

Given the complicated kinetics, the data were re-analyzed using the less-restrictive Maximum Entropy Method (MEM), where the data are considered without a predetermined superposition of components. Data reveal a broad distribution of tryptophan conformations without Q (Fig. 2, red curve). Instead of maintaining a fixed position, evidently the tryptophan can move fairly freely within pure HSA.

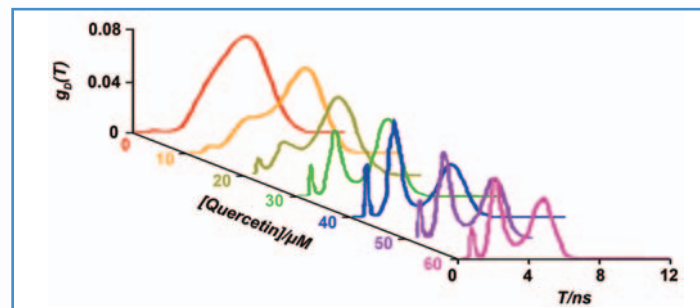


Figure 2: Lifetime distribution function for HSA, with varying [Q] (color-coded). $\lambda_{exc} = 295$ nm.

When Q is added, three lifetime peaks suddenly appear at 1.25, 3.34, and 6.25 ns, because the Q changes the HSA's structure, fixing the tryptophan in certain rotamer conformations. As the concentration of Q rises, lifetimes separate and shorten, resulting from restricted tryptophan movements and more FRET quenching. At the highest Q concentrations, lifetimes decrease further. Perhaps here quenching predominates over structural changes. Comparison of the tri-exponential model with the free-form MEM shows that they are qualitatively similar for HSA-Q binding. The MEM is more sensitive to environmental and structural changes in the complex. Our 5000U lifetime spectrofluorometer with TCSPC is an integral part of researching structure-property relations during protein-binding studies for the biochemical and medical fields.

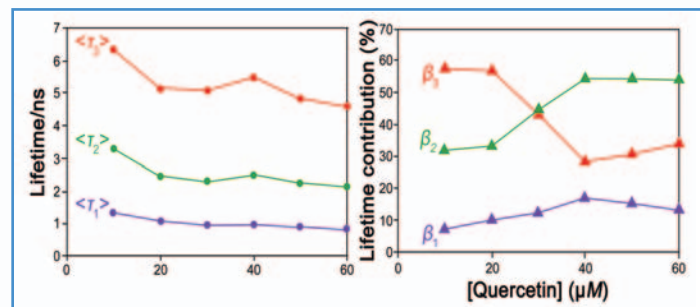


Figure 3 : Results from MEM analysis. Left: Change in each HSA lifetime with [Q]; right: change in relative contribution for each corresponding rotamer.

¹ C.D. McGuinness, et al., "Selective excitation of tryptophan fluorescence decay in proteins using a sub-nanosecond 295 nm light-emitting diode and time-correlated single-photon counting," Appl. Phys. Lett. **86** (2005), 261911–3.

Label-free and real-time biomolecules interaction detection

Chiraz Frydman, HORIBA Jobin Yvon S.A.S., Chilly Mazarin, France

The set-up of the SPRi-Lab+™ and SPRi-Plex™ is based on the Surface Plasmon Resonance imaging principle. We use a surface evanescent wave (plasmon wave) to analyse the interactions between immobilised molecules (probe molecules) on a surface (a thin gold layer in our case) and mobile molecules in a liquid media (target molecules) which can interact with the probe biomolecules.

This technique enables:

- Rapid quantification and monitoring of biomolecular interactions in real-time.
- The study of a series of interactions in multiplex system (up to 400 spots), enabling simultaneous measurements of all reactions to be observed on a biochip surface throughout the entire analysis.
- The following of label-free binding

In a single experiment different parameters can be studied and compared: association / dissociation rates, affinity constant, surface coverage (pg/mm², pmol/mm²...)... In multiplex array format, these parameters can be studied for all surfaces (up to 400) grafted on the biochip.

Applications range from the monitoring of DNA:DNA, protein:DNA, protein:ligand or peptide:ligand interactions in the fields of drug discovery process, early diagnosis, biosecurity, therapeutic antibodies, etc.

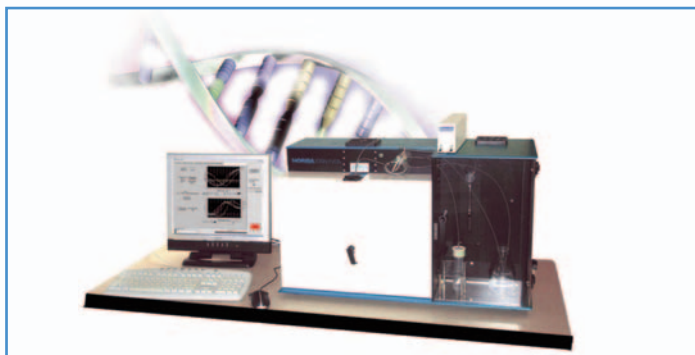


Figure 1: The SPRi-Plex automated solution for multiplex analysis

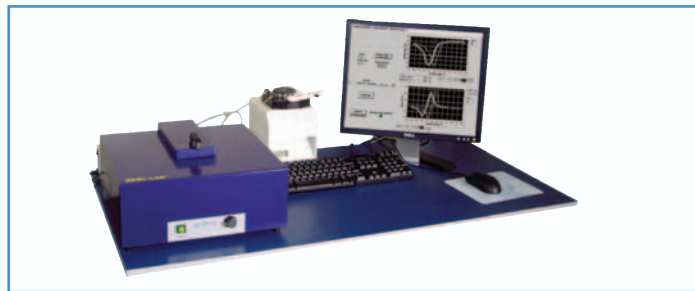


Figure 2: The SPRi-Lab+ useful to develop your own Label-free bioassays

The Biochip:

SPRi-Biochips are chips designed to be used with SPRi-Plex and SPRi-Lab+. They are composed of a high refractive index glass prism coated with a thin gold layer.

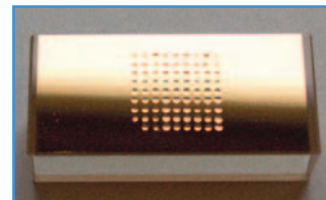


Figure 3: Spotted SPRi-Biochip™

Spotting can be performed using existing spotter equipment. We also offer the possibility of performing electrodeposition using the SPRi-spot manual spotter or, for more demanding applications, via the modification of some commercially available microarrays. With the appropriate regeneration procedure, spotted SPRi-biochips can be reused at least 30 times.

Simple operation enables fast and efficient bio-mechanism studies to be performed with ease. Simply introduce the spotted SPRi-Biochip™ into the SPRi system, add target solutions into the flow cell via a pump and watch the progress of the experiment in real-time.

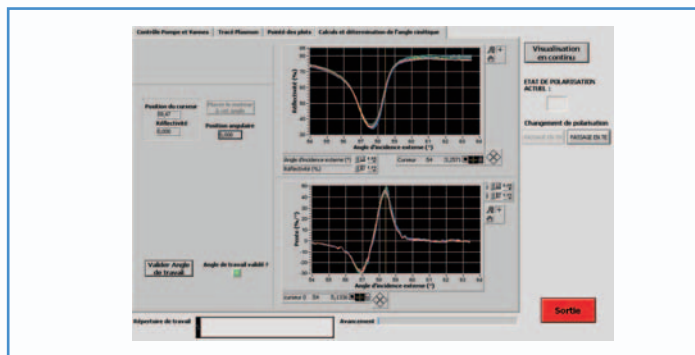


Figure 4: Plasmon curves of several protein spots with SPRi-ViewIt™ software

Micro-XRF investigates bone healing

Simon FitzGerald¹ & Atsushi Bando², ¹HORIBA Jobin Yvon Ltd., Stanmore, UK, ²HORIBA Ltd, Kyoto, Japan

The XGT-7000 x-ray fluorescence micro-analyser is used across a very wide range of disciplines, offering fast, non-destructive elemental analysis with 10 µm spatial resolution. As a result researchers within forensic science, geology, materials, electronics, pharmaceuticals, archaeology and metallurgy all benefit from its extensive capabilities.



Figure 1 : The XGT-7000 system showing the chamber open and sample on the stage.

One unique feature of the XGT systems is the Localised Vacuum Mode, which allows samples to be analysed at atmospheric pressure, whilst retaining sensitivity for the light elements. Signal from these elements are absorbed by air, and the traditional solution for XRF is to analyse the sample in a vacuum. Whilst this is a valid solution for many samples, biological materials cannot be analysed in this way, since the vacuum will quickly dehydrate and damage them. The Localised Vacuum Mode is thus ideally suited for XRF analysis of such samples.

One example of research where this capability is vital is the analysis of bone fracture healing in a frog thigh bone. Understanding of bone healing is necessary for treatment of the many millions of fractures suffered by humans throughout the world.

Once a fracture occurs, there are a number of processes which are initiated for healing, ranging from initial reaction to the fracture (eg, blood clotting) through to regeneration and growth of the bone. Within hours of the fracture fibroblasts replicate to form a structure for new bone growth – after several days, other cells begin the process of new bone growth, forming a callus around the fracture site. Initially the callus overgrows, but with normal use of the bone the callus will typically reduce where possible.

A spectrum taken from the bone is shown in Figure 2, and indicates the expected major elements – calcium and phosphorus from bone's inorganic component, hydroxyapatite.

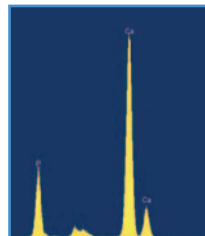


Figure 2 : typical spectrum acquired from the frog bone showing calcium and phosphorus as the major constituents.

X-ray mapping over the bone allows the bone structure and chemistry to be more clearly observed. The transmitted x-ray image clearly illustrates the positioning of the original bone, and the subsequent callus formation around the fracture site. The fracture appears to be clean, but is strongly displaced, hence the significant callus size. The high calcium/phosphorus concentrations within the callus are shown in the XRF image.

Elemental analysis of biological samples such as this would not be possible without the XGT systems. The spatially resolved, non-destructive elemental analysis they provide allows researchers to more fully understand their samples.

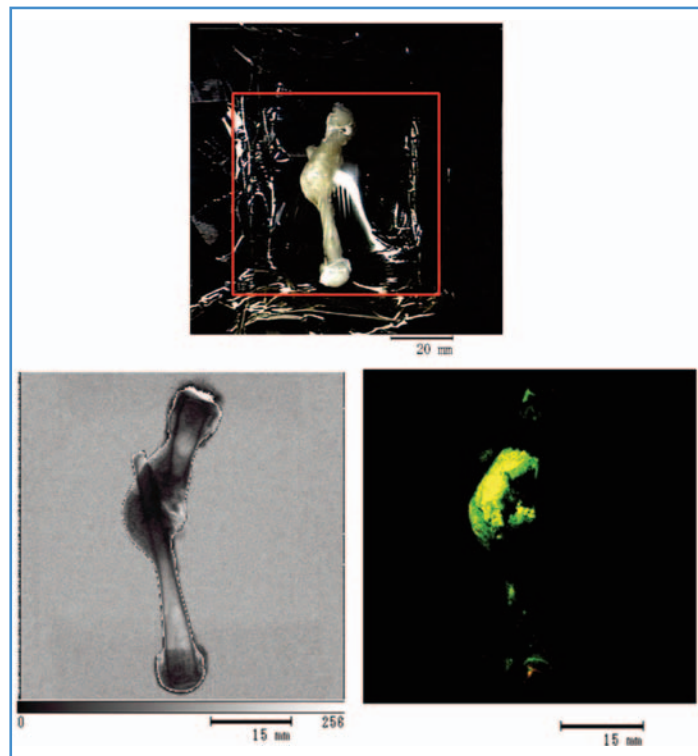
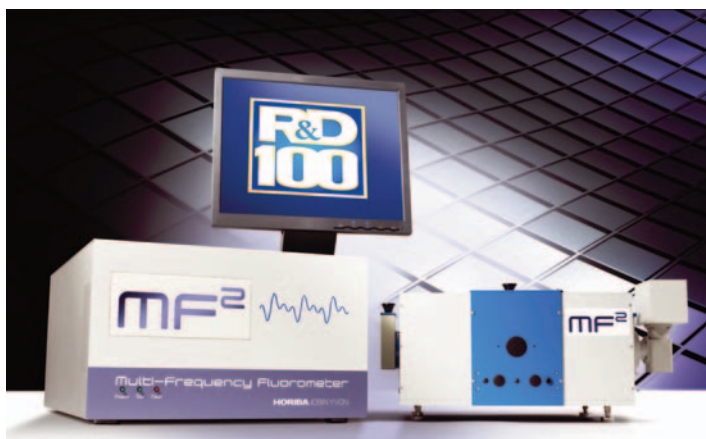


Figure 3 : (left) optical image of frog thigh bone with mapping region indicated, (middle) transmitted x-ray intensity distribution and (right) composite XRF image showing phosphorus (P), calcium (Ca) distribution. Note: rendition of red and green is yellow.



Innovative Instrument for Fluorescence Dynamics Wins R&D 100 Award



The MF² (Multi Frequency Fluorometer), the latest instrument from the Fluorescence Group at Horiba Jobin Yvon, has been named the winner of the R&D 100 Award, which has been described as the Oscar of Invention by the Chicago Tribune.

This award is sponsored by R&D Magazine. And with this award, MF² joins such prestigious products as anti-lock brakes, the fax machine, ATM, liquid crystal displays. The MF² is an instrument using patented technology to instantaneously acquire data on the molecular behavior of substances such as cells, nanodevices, and research materials. As such it is orders of magnitude faster than the nearest competing device and opens up new frontiers in research science.

Find out more, visit our website www.jobinyvon.com

Contact Details

For further information on any of the articles within this newsletter, or should any of your colleagues wish to be part of our mailing list, or should you have any queries or comments, please contact mma-info@jobinyvon.com, or any of the following offices :

FORTHCOMING EXHIBITIONS

16th - 18th October 2007

Lab 2007

Lillestrom, Norway

18th - 23rd October 2007

BCEIA

Beijing, China

27th - 31st October 2007

Geological Soc. America (GSA)

Denver, CO, USA

11th - 15th November 2007

EAS

Somerset, NJ, USA

26th - 30th November 2007

MRS Fall Meeting

Boston, MA, USA

17th December 2007

INSERM

Paris, France

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