Molecular and Microanalysis

Winter 2007-2008

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.

The Molecular & Microanalysis team wishes you and your family a very prosperous 2008!

What's inside:

10 years as part of HORIBA

2007 has marked the 10th anniversary of HORIBA Jobin Yvon being part of the Analytical business of HORIBA, offering analytical and scientific equipment.

The international company, HORIBA, offers a large variety of products for research and development, process control and quality management in industry with four main business areas:

- Automotive Test Systems
- Analytical Systems:
  - Process and Environmental
  - Scientific instrumentation
- Medical Diagnostics
- Semiconductor Applications

Currently employing more than 4500 people worldwide, and with annual sales in excess of one billion dollars, HORIBA is a forward looking company.

Our motto "Joy and Fun" helps us to enjoy exploring new technologies to serve industry and society while keeping an eye on the environment and welfare of mankind.

Tracking protein unfolding on the MF²

Jim Mattheis, HORIBA Jobin Yvon Inc., Edison, NJ, USA

Proteins are important biomolecules that are key to all organisms. Although complex in structure and function, much is known about proteins as a result of many years of fluorescence characterization.

Proteins are unique in that they contain intrinsic fluorescent amino acids the most important being tryptophan. In fact an instrument such as the new HORIBA Jobin Yvon MF² can be used to selectively elicit an observable fluorescence signal from tryptophan and rapidly track changes in the fluorescence as the three-dimensional shape of a protein is altered by environmental interactions such as substrate binding, temperature changes or chemical interactions. When these structural changes are rapid it is important that the signal acquisition time is as short as possible.

The MF² acquisition times can be as short as 1 ms and still retain excellent resolution and accuracy. I describe an important type of experiment that follows the increase in disorder of a protein as a result of increasing temperatures.

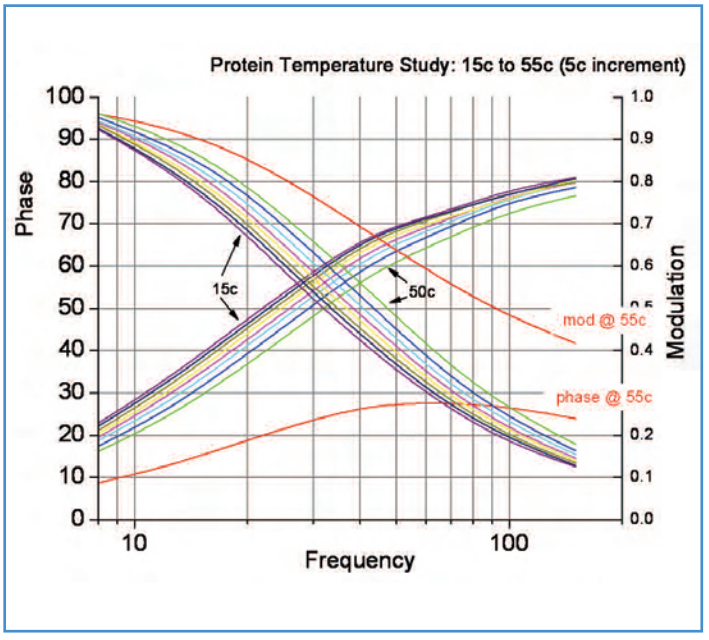

Figure 1: Phase and modulation were measured every 5 degrees from 15 to 55 °C. The small changes up to 50 degrees indicates only subtle changes in the three dimensional structure. The dramatic shift between 50 and 55 °C indicates a complete disordering of the protein.

The intrinsic fluorescence of tryptophan is used to reveal first subtle changes and then extreme changes in the protein three-dimensional structure.

A small quantity of a protein in solution was placed in the MF² standard thermostated sample chamber. Tryptophan fluorescence was elicited using a 295 nm ModuLED. The temperature was programmed to ramp from 15 °C to 55 °C while rapidly measuring the fluorescence lifetime of tryptophan. For experimental results refer to figure 1 and table 1.

Table 1. shows the changes in the lifetimes of the tryptophan as the temperature increases:

Temp °C	Tau1 ns	Fractional intensity	Tau2 ns	Fractional intensity	Tau3 ns	Fractional intensity	Chi^2
15	8.689	0.995	0.342	0.005			1.270
20	8.381	0.995	0.347	0.005			2.030
25	8.032	0.995	0.144	0.005			1.680
30	7.689	0.994	0.532	0.006			1.770
35	7.332	0.995	0.218	0.005			1.110
40	6.933	0.996	0.557	0.004			1.080
45	6.486	0.995	0.139	0.005			0.954
50	6.005	0.987	0.436	0.013			1.020
55	5.096	0.622	0.147	0.378			2.650
55	5.820	0.499	0.100	0.353	2.450	0.149	0.953

Because of the HORIBA Jobin Yvon MF² revolutionary acquisition speed and versatility it is becoming a valuable tool in the understanding of protein structure and function.

Figure 2: The HORIBA Jobin Yvon MF²

For further information on Fluorescence applications, please visit our website www.jobinyvon.com/fluorescence/applications

Polarized Raman spectromicroscopy of spider silk

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Spider silk is among nature's most highly engineered structural materials, achieving, in some cases, combinations of strength and toughness that are almost unmatched by other high-performance synthetic fibers. Spiders produce several different types of silk with a specific range of mechanical properties that are used for a specific or multiple functions.

Dragline silk is a particularly high toughness fiber that combines both stiffness and extensibility. Such properties are due to the peculiar block copolymer structure of dragline silk that is composed of alternating alanine-rich hard segments containing β-sheets and glycine-rich soft segments. The alignment of the β-sheets within the fiber is an important factor responsible for the spider dragline silk tensile strength. To establish structure-property relationships in spider silk, it is of fundamental importance to quantify the level of orientation and the conformations in silk fibers.

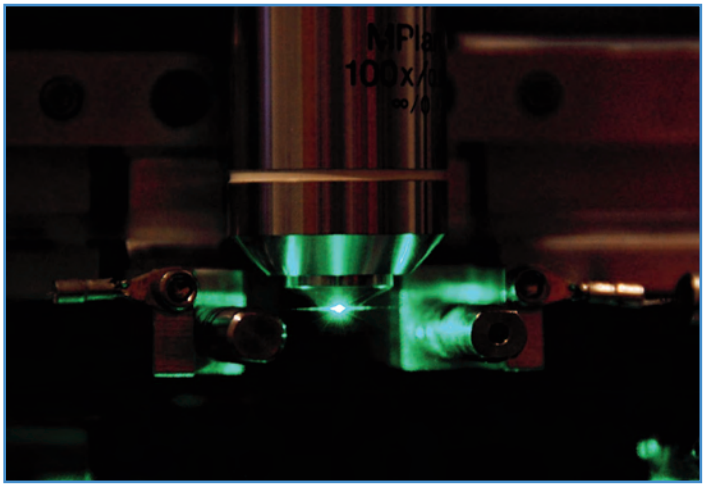

Figure 1: Fiber irradiated by a laser beam

Raman spectromicroscopy is a powerful technique to achieve this goal. Its main advantage is that the laser beam can be focused to about 1 µm in diameter in the sample, therefore allowing the *in situ* recording of high quality spectra of single native silk filaments. Polarization measurements provide quantitative information about the orientation of the secondary structure elements in the samples.

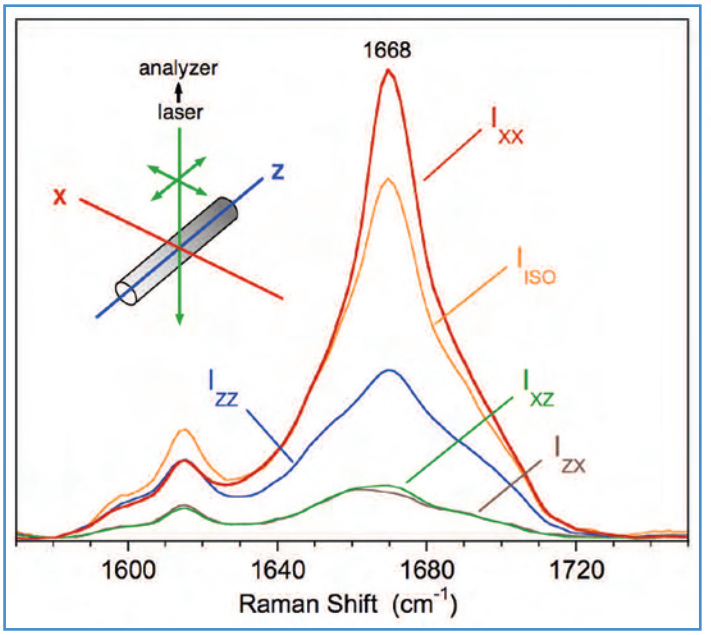

Figure 2: Polarized Raman spectra of a Nephila clavipes *fiber for the four polarization configurations where the first and second letters represent the laser and scattered light polarization, respectively, and isotropic spectrum calculated from the polarized spectra.*

A typical example of polarized Raman spectra in the amide I region of a dragline monofilament of the *Nephila clavipes* orb-weaving spider is shown in Figure 2. As seen, the 1668 cm^{-1} amide I band essentially due the β-sheet conformation is much stronger in the I_{XX} spectrum than in the I_{ZZ} spectrum, thus showing that the carbonyl groups are highly oriented perpendicular to the fiber axis and that the β-sheets are oriented parallel to the fiber axis. This conclusion was confirmed by the quantitative analysis of the polarized spectra as described previously [1]. Values of -0.34 and 0.16 were calculated for the order parameters $\langle P_2 \rangle$ and $\langle P_4 \rangle$, respectively, the value of $\langle P_2 \rangle$ for perfect perpendicular orientation being -0.5. Figure 2 also shows that the shape of the amide I band is polarization dependent. To obtain more information about the protein conformation in oriented silk, the orientation-insensitive spectrum presented in Figure 1 was calculated from the four polarized spectra [2]. The band fitting of this spectrum has shown that the β-sheet content of *N. clavipes* dragline silk is about 37%, in agreement with the amino-acid sequence of silk proteins.

1. M.-E. Rousseau, T. Lefèvre, L. Beaulieu, T. Asakura, M. Pézolet, 2004. Study of Protein Conformation and Orientation in Silkworm and Spider Silk Fibers Using Raman Microspectroscopy, Biomacromolecules, 5, 2247-2257.
2. T. Lefèvre, M.-E. Rousseau, M. Pézolet, 2006. Orientation-Insensitive Spectra for Raman Microspectroscopy, Appl. Spectrosc., 60, 841-846.

Real time monitoring of protein-peptide interactions by Surface Plasmon Resonance Imaging

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[2]CREAB, UMR 5819 (CEA, CNRS, UJF), CEA Grenoble, France.
[3]Département d'Hépato-Gastroentérologie, CHU de Grenoble, Grenoble, France.

Proteins constitute the essential machinery in cellular life; almost all cellular responses are triggered by protein/protein interactions. Performing assays to specifically analyze binding phenomena is important for understanding cellular mechanisms and for designing pharmacologic targets. These events cannot be studied with RNA/DNA chips because RNA concentrations do not always correlate with protein production and/or activity.
Protein array techniques have recently emerged but these arrays remain difficult to construct because of the complexity and heterogeneity of proteins and the necessity to preserve their conformational folding after covalent binding to a surface.

Microarray technology has a crucial role to play in detecting molecule-molecule interactions. The use of peptide chips with indirect detection by use of labelled probes allows qualitative analyses of samples but fails to provide quantitative data such as kinetics.
Optical detection by surface plasmon resonance (SPR) is an accurate, one-step method for the real-time direct measurement of ligand binding without labelling. However, this approach does not allow for parallel analysis on a single chip bearing an array of proteins or peptides. For this it is necessary to use SPR imaging (SPRi).

Analysis of patient-derived sera by use of peptide chips and SPRi detection

Eleven peptides derived from hepatitis C virus (HCV) and one peptide from ovalbumin (negative control) were synthesized with a maleimide-modified NH_2 terminus and were conjugated with SH-activated pyrrole. Then, the pyrrole-peptide conjugates were electro-deposited on the gold layer of the chip. The biochip was then introduced in the SPRi-Plex™ platform in order to study peptides-circulated antibody interactions in complex media such as undiluted sera using SPRi.

Eleven sera samples from HCV-positive patients and from healthy donors were successively injected on a chip bearing 11 HCV peptides. The specific SPRi signal (ΔR) varied according to the patient and the peptide, revealing donor-related differences in the anti-HCV antibody responses (specificity, amount and affinity).

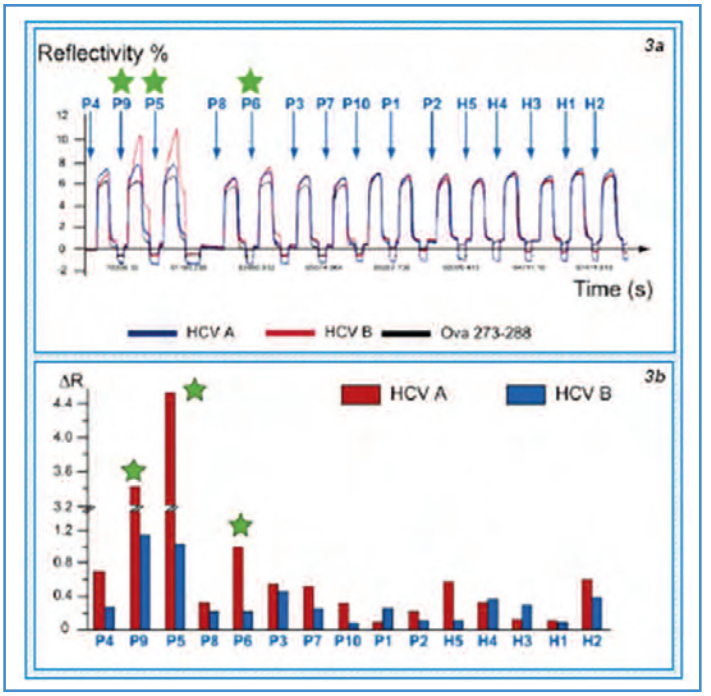

Figure 1: SPRi analysis of sera from 10 HCV patients (P1-P10) and from 1 healthy donor (H1). Injection of human sera on a chip bearing 11 HCV peptides and Ova (negative control). The chip was regenerated between 2 successive injections. 3a: Interaction curves obtained after injection of the 11 sera samples. 3b: ΔR for each serum.

Conclusion

The SPRi platform enables:

- The generation of biochips bearing a large range of peptide probes (peptide arrays)
- The label-free detection of peptide-antibody interactions in complex media such as undiluted sera

The SPRi technology is a powerful tool for clinical applications such as diagnosis, pharmacologic studies and for research.

Application note courtesy of Genoptics

Micro-XRF analysis for lead contamination in toys

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Environmental concerns are becoming increasingly stronger, and regulatory bodies across the world are beginning to impose strict guidelines affecting manufacturers and importers for a very wide range of products. More recently, there have been growing concerns over the presence of harmful materials within toys.

To a child a toy is an object which could be held for extended periods, put in the mouth, and even swallowed (albeit unintentionally). Hence it is paramount that toys are made with materials which present no health risk. In 2007 there were growing concerns about imported toys within America, many of which have been shown to contain dangerously high levels of lead. This can cause a number of serious health problems, including learning disabilities, kidney failure, anaemia and even irreversible brain damage. Many millions of toys were recalled, and it is clear that manufacturers, importers and government laboratories require a fast, reliable analysis method to check for high levels of harmful elements.

X-ray fluorescence micro-analysis provides just such a solution. Within a typical analysis time of 30s it is possible to quickly ascertain not only what elements are present (ranging from sodium to uranium), but also their concentrations (at % and ppm levels).

Figure 1: The toy used for analysis, showing the specific region of concern before and after partial dismantling.

A suspect toy (Figure 1) was analysed for elemental composition in a number of regions. Whilst most points revealed zero or negligible concentrations of lead, there was one area which gave significant cause for concern. By partially dismantling the toy it was possible to more fully interrogate this concern area. The toy, a plastic robot-machine with movable limbs and weapons, includes caterpillar tracks composed of a black polymeric material. Spot analysis (Figure 2) on the left hand track shows a very low concentration of lead (52 ppm), which is well within the limit of 600ppm suggested by the USA's Consumer Product Safety Commission (CPSC). However, the other track reveals a lead content of 3000 ppm, which would present a serious health threat to a child.

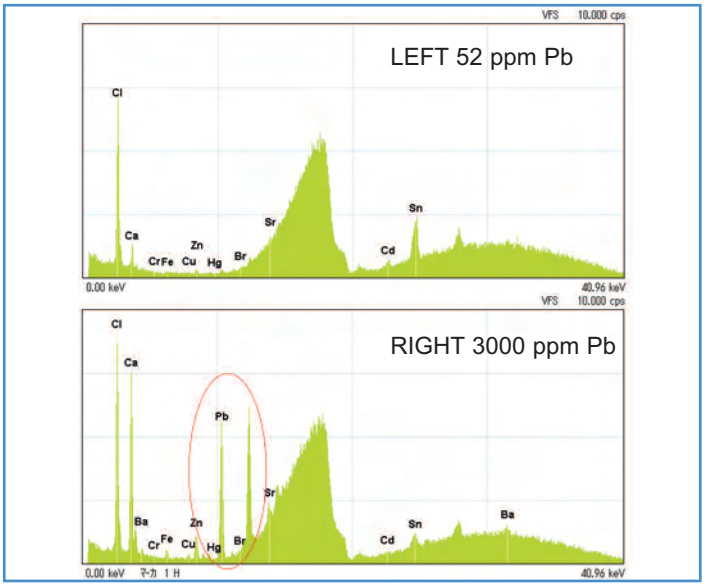


Figure 2: XRF spectra acquired from the left and right caterpillar tracks.

A low resolution XRF image (Figure 3) acquired over the caterpillar tracks clearly illustrates the difference in composition of the two tracks – the one on the left shows negligible lead content, whilst the right hand track has a uniformly high lead content.

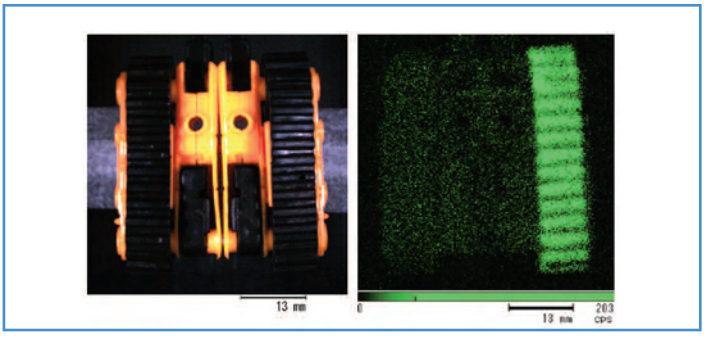
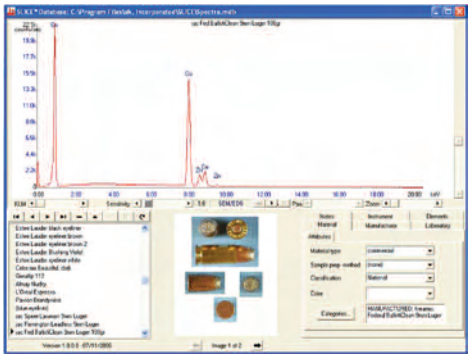


Figure 3: Optical image (left) and low resolution XRF image (right) showing the distribution of lead in the caterpillar tracks.

SLICE : A New XRF Methodology and Library for sample Identification and archiving

HORIBA Jobin Yvon is pleased to announce that it has reached an agreement with xk, Inc. to become the exclusive provider of SLICE software to the XRF community. SLICE, the Spectral Library Identification and Classification Explorer, is designed to archive, query, and compare x-ray spectra, and represents a revolutionary new approach to materials analysis. It was developed under an FBI contract to save time and improve the accuracy of evidence identification, and to create an advanced archiving tool, allowing spectra, images, and text to be seamlessly linked. Searching the database allows the spectra to be interrogated quickly and efficiently. It has become a widely-used tool within Forensic laboratories where virtually any material can be encountered, and its use is now being extended to contaminant analysis in a wide range of industries, including pharmaceutical, automotive, engine wear and semiconductor. HORIBA Jobin Yvon's XGT micro-XRF analysers are used across these fields, and in combination with SLICE offer the analyst a complete solution for materials identification.



FORTHCOMING EXHIBITIONS

7th - 9th January 2008
CHI-Pep Talk
San Diego, CA, USA

19th - 24th January 2008
SPIE Photonics West
San Jose, CA, USA

24th- 25th January 2008
Tip Enhanced Raman & Fluorescence Spectroscopy (TERFS)
Teddington, UK

19th - 21st February 2008
Conferences French Institute
Budapest, Hungary

25th - 29th February 2008
DPG Jahrestagung
Berlin, Germany

1st-7th March 2008
PITTCON
New Orleans, LA, USA

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

Contact Details

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