Molecular and Microanalysis

Summer 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 next at:

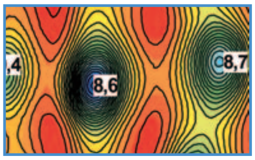
JAIMA 2007

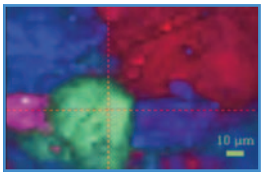
29th - 31st August, Japan

What's inside:

Page 2: Development of unique SWNT species synthesis methods using the NanoLog®

The NanoLog® near-IR spectrofluorometer provides rapid qualitative and quantitative data on single-wall carbon nanotubes with a superb signal-to-noise ratio.



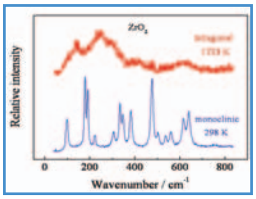


Page 3: Single Point Analysis and Raman Mapping of Tablet Dosage Formulation as a Means for Detecting and Sourcing Counterfeit Pharmaceuticals

Raman and FTIR microscopy are being used to identify counterfeit tablets entering the drug delivery stream by chemical mapping of the API and excipients.

Page 4: A New Dimension in Ultra High Temperature Raman Spectroscopy

The LabRAM HR Raman microscope coupled to an ICCD (Intensified Charge Coupled Device) detector and pulsed laser source has provided enhanced *in-situ* Raman spectra at high temperatures.



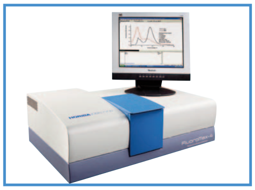
Page 5: Innovation in XRF with the XGT-7000

The XGT-7000 x-ray fluorescence system provides a new dimension in elemental micro-analysis, offering analysis spot sizes down to 10 micrometers with both full vacuum and localised vacuum sampling modes.

Page 6: FluoroMax®-4

The new bench-top spectrofluorometer

Page 6: Our next conferences and shows



In-situ Raman Spectroscopy in the World's Oceans

M. LeHaitre, IFREMER, and M. Leclercq, HORIBA Jobin Yvon S.A.S., in collaboration as part of European Projects.

Raman Spectroscopy is no longer exclusively a laboratory analytical technique. It has become a very promising way for chemical and environmental monitoring in the undersea world.

Increasing concern over the natural environment has prompted regulatory bodies to enforce continued monitoring of coastal areas and estuaries. To meet this challenge, it has been necessary to develop marine equipment to monitor accidents and spills, act as alarm sensors and support oceanographic studies.

Marine Raman spectroscopic analysers have been designed for underwater operation in any marine or deep sea environment from shallow estuaires to deep water trenches.

Visit www.jobinyvon.com/oceans to find out more.

Remote-controlled Raman measurements at sea from towed vehicle and a ROV.

Development of unique SWNT species synthesis methods using the NanoLog®

Adam Gilmore and Stephen Cohen, HORIBA Jobin Yvon Inc., Edison, NJ, USA

The NanoLog® spectrofluorometer, specially optimized for recording near-IR fluorescence from nanoparticles has optimal excitation optics for single-wall carbon nanotube (SWNT) research, which may find useful applications in electronics, biosensing, and diagnostic tools for medicine.

Corrected emission spectra of carbon nanoparticles can provide excitation-emission matrices (EEMs) for a range of excitation wavelengths within minutes. EEM data (Figure 1, solid lines) and simulations (contour-maps) from two SWNT suspensions from two different manufacturing processes are distinguished in Figure 1 by their different size and helical distributions. The high-pressure carbon-monoxide method (HiPCO, Figure 1A) forms many sizes, helical angles, and species of SWNTs. The cobalt-molybdenum catalytic method (CoMoCAT, Figure 1B) has a narrower average size and smaller helical-angle distribution. Figure 1A identifies five main HiPCO species while Figure 1B identifies four main CoMoCAT species in the specified regions. Using our unique Nanosizer® SWNT analysis software (patent pending), Figure 1C compares species found in Figures 1A and B, and plots the helical angle versus SWNT diameter against intensity of emission (symbol size/color).

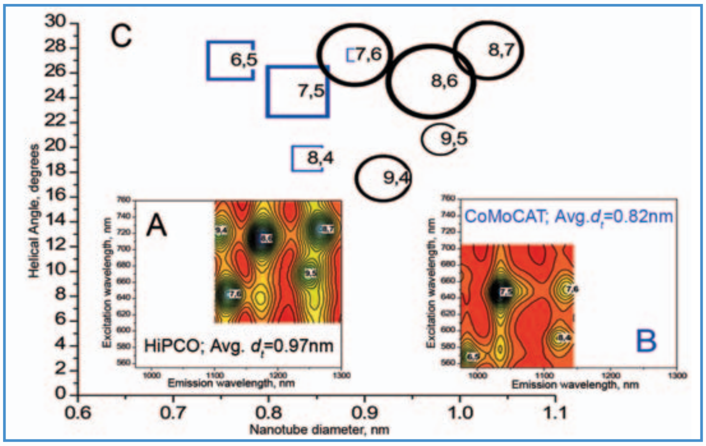


Figure 1: EEMs (A and B) and helical (C) maps of HiPCO and CoMoCAT SWNT suspensions. Solid lines (A and B) are data; color contours are simulations. Symbol sizes (C) show relative amplitudes for HiPCO (circles) and CoMoCAT (squares), each normalized to 1.

High signal-to-noise ratio (*S/N*) is crucial to unambiguous, rapid quantitative determination of the multiple species present in SWNT samples. To show the NanoLog®'s high *S/N*, we studied a sample of HiPCO SWNTs — a broad size-distribution, with many helical angles and species. Figure 2 shows an emission spectrum (a) with known peaks, and an excitation spectrum (b) of noise. Both spectra were corrected for source inhomogeneities and dark noise.

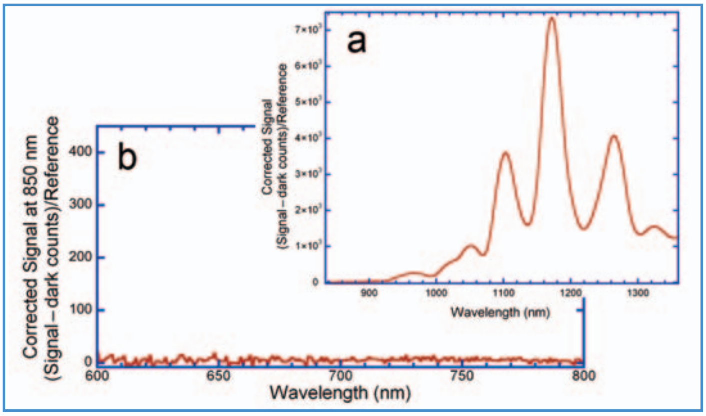


Figure 2: Emission spectrum [plot (a)] of HiPCO SWNTs, and excitation spectrum [plot (b)]. Spectra are corrected for excitation inhomogeneities (via the reference detector) and dark counts. Plot (b) was used to calculate noise.

With $S/N = (S_{peak} - S_{background})/(S_{background})^{1/2}$, the maximum signal was 7357.41 at 1171 nm, and the average noise was 5.443, giving a $S/N = 3151$. This very high *S/N* is not achievable on other systems, and enables accurate, unambiguous determination of SWNT mixtures. Dr. Yuan Chen, at Nanyang Technological University in Singapore, has been investigating selectivity of SWNT synthesis via varying the carbon precursors for the CoMoCAT method, using the NanoLog®. He has achieved a narrow distribution of chiralities, as shown in Figure 3, with high-pressure CO or alcohol under vacuum (5 mbar), as the precursors. Chirality selectivity can be changed under different precursors. Specific chiral angles are color-coded to the EEMs.

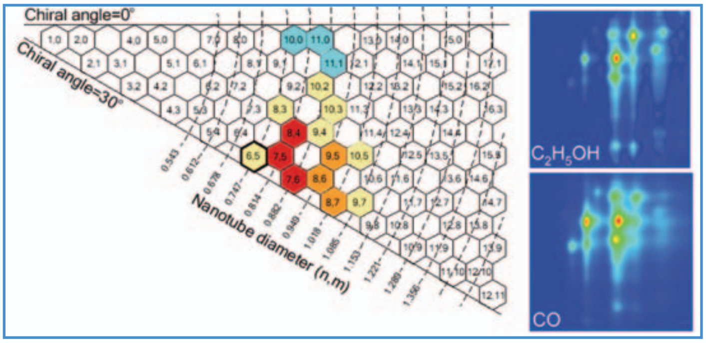
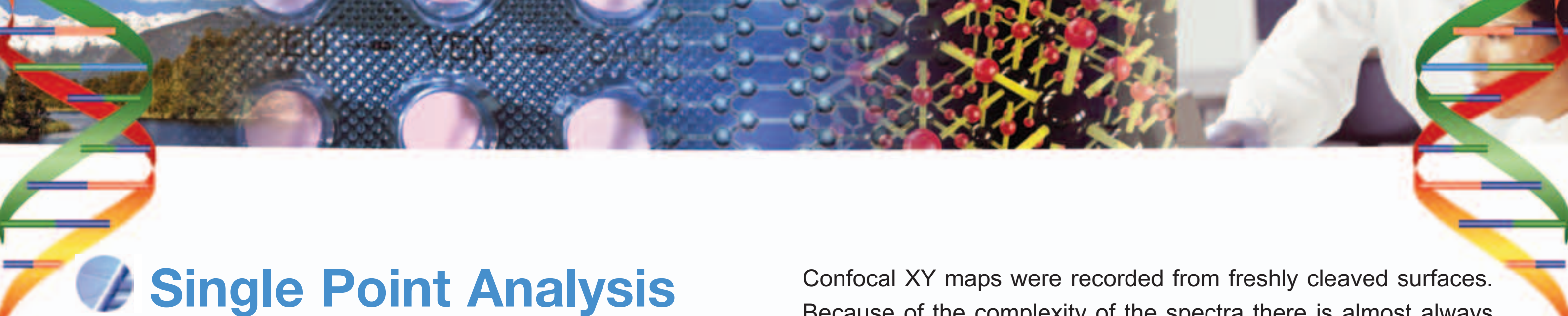


Figure 3: Chiral map of nanotube structures produced by Dr. Chen's methods. Red and orange indicate predominant SWNTs. EEMs of CoMoCAT SWNTs using CO and ethanol precursors respectively are also shown.



Single Point Analysis and Raman Mapping of Tablet Dosage Formulation as a Means for Detecting and Sourcing Counterfeit Pharmaceuticals

Mark Witkowski [1], Fran Adar [2],

[1] US Food and Drug Administration's Forensic Center, Cincinnati, OH, USA, [2]HORIBA Jobin Yvon Inc., Edison, NJ, USA

The development of methods to rapidly differentiate counterfeit pharmaceuticals from authentic products is one of the ways to prevent these products from entering the drug distribution chain. The capability of vibrational spectroscopy to identify the molecular and crystalline phase of almost any material has been exploited to characterize suspected counterfeit pharmaceutical tablets by combining spectroscopy with spatial mapping and multivariate analysis of Raman maps. Single point Raman and FTIR spectra, and maps of authentic and counterfeit tablets effectively demonstrate which seized suspect products are counterfeit that could not come from legitimate manufacturers, a conclusion derived from the identification of the excipients and from their distribution. This work has added importance because samples are not destroyed before or during analysis, making them available for further investigations.

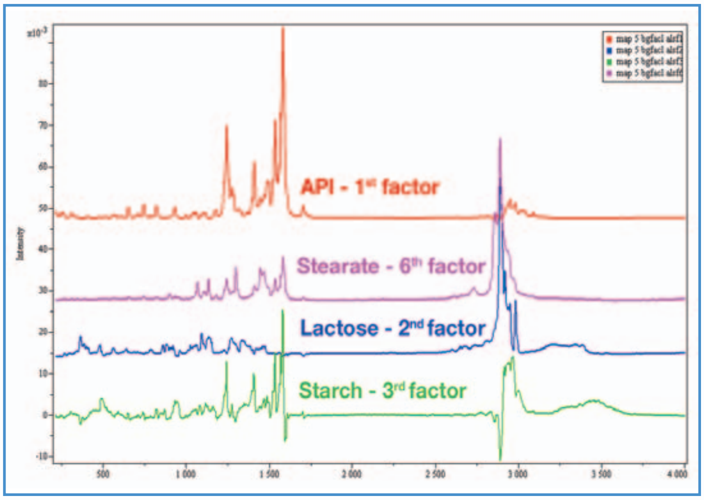


Figure 1: Loadings of the API and 3 excipients extracted by Multivariate Analysis.

Confocal XY maps were recorded from freshly cleaved surfaces. Because of the complexity of the spectra there is almost always spectral overlap. Multivariate techniques, especially Factor Analysis with Alternating Least Squares, Score Segregation and Binary Rotation, enable one to spatially isolate contributions from the different species. The high spatial resolution of the Raman technique also makes it easy to detect the presence of materials such as Mg stearate that are present at low levels.

Results from the suspect tablets are shown. The first figure shows the "purified" loadings generated through Isys™, data is easily passed between LabSpec and Isys™, in both directions, using direct icon selected links. These loading spectra are then used by the modelling function in LabSpec to create the multicolored image in the second figure. Note that all all the particles in this figure are 20 µm or greater.

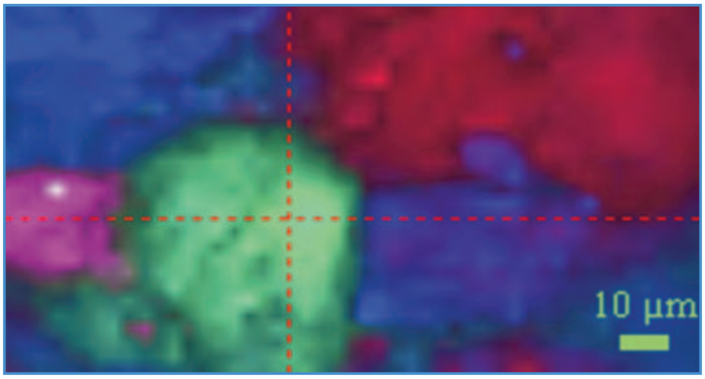


Figure 2: Raman image created by the modeling function in LabSpec, using the factor loadings derived by Isys™.

This image shows large amounts of starch (not present at all in the authentic tablet) and large amounts of lactose (more than what was observed in the authentic sample).

Products of the innovator companies are consistently well dispersed in the manufacturing process, which is not not always the case with counterfeit products and Raman mapping is one method which allows for the excipient particle size and dispersion to be assessed. In this example Mg stearate, a component that is present at low concentrations was detected and this can be quite important because Mg stearate is added as a lubricant, but can inhibit bioavailability of the API if it encapsulates the API or dispersants.

These results are part of an ongoing collaboration between HORIBA and Mark Witkowski at the US Food and Drug Administration's Forensic Chemistry Center in Cincinnati, Ohio.

The mentioning of specific products / instruments in this presentation is for information purposes only and does not constitute an endorsement by either the Food and Drug Administration and / or the Forensic Chemistry Center.

A New Dimension in Ultra high temperature Raman Spectroscopy

By You Jinglin, Jiang Guochang, Chen Hui, Wu Yongquan and Xu Kuangdi, Shanghai University

A new configuration for the LabRAM HR system targeted at ultra high temperature measurements has been constructed by Shanghai University and HORIBA Jobin Yvon.

With high temperature measurements there is often the need to eliminate the influence of intense thermal backgrounds caused by blackbody radiation. Researchers at Shanghai University have been developing high temperature Raman spectroscopic (HTRS) techniques aimed at greatly enhancing the intensity of the Raman signal above this problematic background radiation. First experiments using a HJY U1000 double spectrometer and a pulsed semiconductor laser enabled exploration of the time resolution method in the early stages. It was possible to study the temperature dependent Raman spectra (up to 2023 K) of various inorganic materials including silicates, borates, phosphates, carbonates and zirconia even in their molten states.

The next step was to develop the method on a modern confocal Raman microscope, LabRAM HR, and to use the combination of a pulsed laser and an ICCD detector (capable of single photon sensitivity, and gated in the nanosecond realm). The intensifier of the ICCD acts effectively as a very fast electronic shutter in this mode of operation and makes it perfect for measurements requiring temporal isolation. It enabled the signal collection to be precisely synchronized with the laser pulse irradiating the sample.

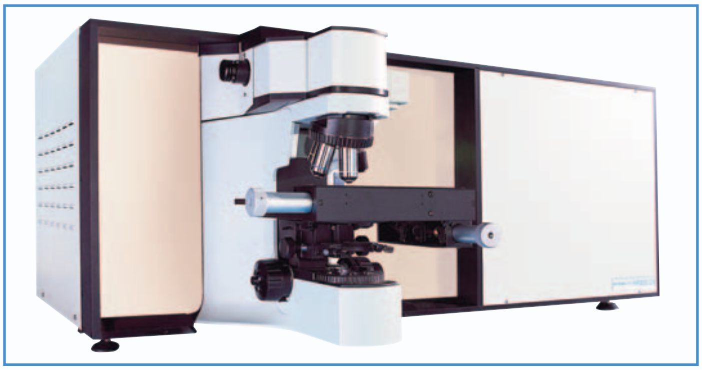


Figure 1: The HORIBA Jobin Yvon LabRAM HR

The pulsed laser emitting at 532nm with a time duration of 10 ns enabled single pulses to be used to excite the sample under investigation. The ICCD detector (based on a Marconi 30-11 sensor) gave the appropriate time resolution for the detection of signals generated from the pulsed irradiation. The result of this technical development is an instrument that combines the potential of both time and spatial resolution.

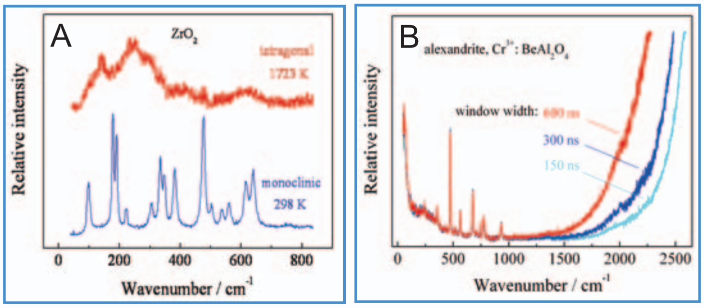


Figure 2A: Room (blue) and high temperature (red) Raman spectra of different phases of ZrO_2.

Figure 2B: Recorded Raman spectra of alexandrite at different acquisition times showing background reduction of time-dependant fluorescence.

An average laser power of 180 mW with 100 s acquisition time were typically used for measurements on samples such as zirconia. Figure 1A demonstrates that the sample of a pure zirconia poly-crystal transformed from the monoclinic phase to the tetragonal phase whilst being heated, and that the temperature of the phase transition was around 1440 K. High quality spectra (without any usual background subtraction) were obtained under high temperature conditions and matched well with previously reported data. It clearly demonstrated that a significant level of thermal radiation background rejection is possible with this method.

A further dimension in time-resolved Raman became apparent in the experiments shown upon another mineral sample, alexandrite. It is difficult to obtain the Raman spectrum of the alexandrite sample, (Cr^{3+}: $BeAl_2O_4$) by using conventional CW visible laser excitation even at room temperature since an intense fluorescence background exists. With the use of the adjustable time gated window available with the ICCD detector, it was possible to obtain clear Raman signals with a far lower spectral background (see Figure 1B). Temporally separating Raman from Fluorescence on a principally standard Raman system is quite an achievement and moves away from the more complex equipment needed for Kerr gated or similar such experiments. This thus has the potential for many interesting Raman analyses where fluorescence backgrounds have been the limiting factor to a successful measurement.

Innovation in XRF with the XGT-7000

Simon FitzGerald, Principal Scientist, HORIBA Jobin Yvon Ltd., Stanmore, UK

X-ray fluorescence is a widely used technique for fast qualitative and quantitative analysis of elemental composition for solids, liquids and powders. However, it has typically been a bulk sampling technique, and often requires destructive sample preparation (eg. grinding into a uniform powder, or fusion within a glass matrix). The XGT-7000 micro-analysis system combines the benefits of XRF with microscopic capabilities, introducing a new era in elemental analysis.

This instrument utilises high intensity x-ray beams with diameters ranging from 1.2 mm down to 10 µm, to provide ground breaking spatial resolution in a compact bench top format. These beams are formed using carefully designed glass capillary x-ray optics - the x-ray guide tube (XGT) - which channel x-rays from an *in-situ* generator into a well collimated, narrow beam.

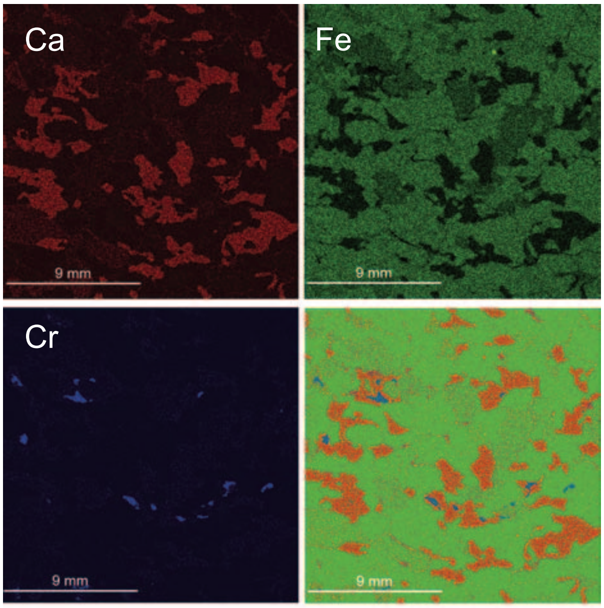
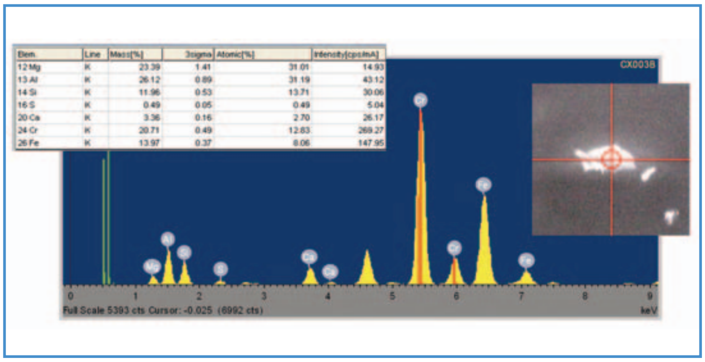


Figure 1: XRF element images showing distribution of calcium, iron and chromium in a mineral section. A composite image of the three elements is shown bottom right.

The resulting high intensity micro-beams allow scientists to analyse individual microscopic particles or features in the matter of seconds - no longer are bulk quantities required, and sample preparation is a thing of the past. Without sample grinding/fusing it is now possible for a sample to retain its natural heterogeneity which can then be probed using the element imaging function of the XGT instruments.

Using a motorised sample stage the XGT-7000 SmartMap software acquires a full spectrum at each and every pixel across a user defined surface area - thus, images showing the distribution of elements across the sample surface can be generated either during or after the acquisition. The optimised beam collimation also allows simultaneous acquisition of transmitted x-ray images, which can be used to study a material's internal structure and to identify features invisible by eye.



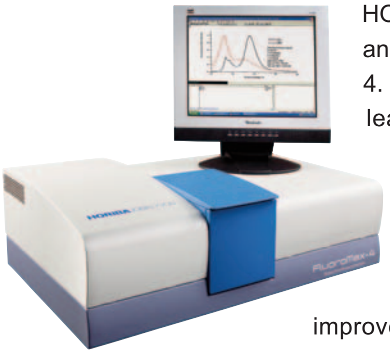
Elem.	Line	Mass[%]	3sigma	Atomic[%]	Intensity[cps/mA]
12 Mg	K	23.39	1.41	31.01	14.93
13 Al	K	26.12	0.89	31.19	43.12
14 Si	K	11.96	0.53	13.71	30.06
16 S	K	0.49	0.05	0.49	5.04
20 Ca	K	3.36	0.16	2.70	26.17
24 Cr	K	20.71	0.49	12.83	269.27
26 Fe	K	13.97	0.37	8.06	147.95

Figure 2: Micro-XRF allows single particles to be pinpointed for analysis yielding both qualitative and quantitative information.

A unique feature of the XGT-7000 is its new dual vacuum mode, which is used to ensure high sensitivity to even very light elements such as sodium and magnesium. XRF signal from these elements is strongly absorbed by air, so a full vacuum can be set in the sample chamber to prevent this. However, many samples cannot tolerate such conditions - for example, biological tissues and cells, and fragile archaeological items. In this case, a localised vacuum can be set in a small sealed unit incorporating the guide tube and detector. The sample remains at full atmospheric pressure, but nonetheless can be analysed for all elements from sodium upwards.

The XGT-7000 provides a fresh approach to elemental analysis, where performance, spatial resolution, size and ease of use are rolled into one unique system. Its capabilities are embraced by scientists from a wide variety of fields, including forensic science, engine wear monitoring, foreign material ID, geology, metallurgy, gemmology, pharmaceutics, biology and electronics.

New FluoroMax®-4 Bench-top spectrofluorometer



HORIBA Jobin Yvon is proud to announce the new FluoroMax®-4. With a superb sensitivity of at least 400,000 cps (a 33% improvement) for the water-Raman peak at 397 nm, and an industry-leading signal-to-noise ratio of 3000:1 minimum (a 20% improvement), the FluoroMax®-4 stands out from the rest. Fluorescence measurements have never been easier in a bench-top spectrofluorometer, with a wide range of accessories and our new FluorEssence™ software for Windows®. Versatile, powerful, and compact are the hallmarks of the FluoroMax®-4. Perfect for basic research, analytic measurements, and quality control, the FluoroMax®-4 uses an ozone-free xenon arc lamp for broadband coverage from the UV to near-IR.

HORIBA Jobin Yvon backs the FluoroMax®-4 with nearly 200 years of sales, service, and applications expertise in optical instrumentation. No one else can come close to The World's Most Sensitive Spectrofluorometer!

Find out more at www.jobinyvon.com/FluoroMax

FORTHCOMING EXHIBITIONS

5th - 9th August 2007
Microscopy & Microanalysis
Fort Lauderdale, FL, USA

19th - 23rd August 2007
ACS Fall Meeting
Boston, MA, USA

29th - 31st August 2007
JAIMA
Makuhari Messe, Japan

21st - 25th August 2007
Regional Biophysics Conference
Balatonfüred, Hungary

1st - 6th September 2007
ECSBM
Paris, France

25th - 28th September 2007
ILMAC
Basel, Switzerland

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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