

Spring 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.

Come & discover the XploRA™ at Pittcon, Analytica and Photonics Europe

## What's inside:

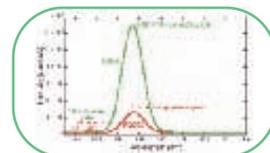
### Page 2: Smart Microscopy with the XploRA™

The experts in Raman from HORIBA Jobin Yvon bring chemical identification to your microscope images with the new XploRA™.



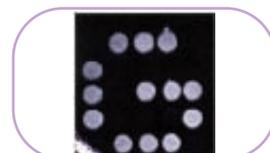
### Page 3: Extending the range of the FluoroMax®-4 into the near-IR

Especially for nanoparticle research, we have extended the range of the FluoroMax®-4 into the near-IR using the R2658P photomultiplier tube.



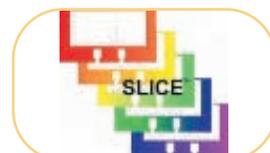
### Page 4: Antibody-Antigen Specific Interaction

The power of the label-free SPRi technology, to analyse a large number of spots at the same time and in the same condition.



### Page 5: SLICE, a database approach to XRF microscopy

Discover the power of the SLICE database for comprehensive XRF data searches and true sample identification. Combining images, spectra and case histories for a robust EDS/XRF identification tool.



### Page 6: HORIBA Jobin Yvon present at TERFS, UK

HORIBA Jobin Yvon attending the TERFS conference on Tip Enhanced Raman and Fluorescence Spectroscopy in January



### Page 6: Our forthcoming conferences and shows

## Celebrating 80 years of Raman Spectroscopy

HORIBA Jobin Yvon celebrate 80 years of Raman spectroscopy with the introduction of the new Raman microscope, the XploRA™.

80 years ago this month, Chandrasekhara Venkata Raman first reported in February 1928 the light-scattering phenomenon that has come to be known as the Raman effect (1). Raman's earliest spectra were recorded with a small prism spectroscope equipped with a photographic plate. The spectra were excited with a mercury arc lamp, using a filter to select the mercury line of interest, and the samples were contained in a large spherical flask.

Since, there have been tremendous improvements through the introduction of laser sources, CCD detectors, and laser filters. Raman equipment now enables routine analysis in a wide range of applications, from pharmaceuticals to mineralogy, polymers to bio-tech, semiconductors and nanomaterials.

HORIBA Jobin Yvon brings to you the XploRA™ which adds chemical identification to your microscope images (see page 2 for more information).



References: (1) CV Raman, KS Krishnan. A new type of secondary radiation. Nature 121.501; (31 March 1928) (cabled to Nature on 16 February)

## The new XploRA™ Raman Microscope

HORIBA Jobin Yvon, the world leaders in Raman spectroscopy, have introduced a new high performance, lower cost Raman microscope. The XploRA™ is a new concept in Raman microscopy bringing Raman chemical identification directly to your microscope.

### Smart Microscopy

The system provides the established performance of HORIBA Jobin Yvon Raman microscopes, combining microscopy and chemical analysis whilst retaining the full functionality of your microscope coupled with high performance Raman spectroscopy. Compact and rugged in design, the XploRA™ is easy to use and transport due to its minimal footprint, making it the ideal smart microscope for every R&D, QA/QC and forensic lab.

Now you can explore the true nature of your sample with rapid compound identification and chemical imaging, with no sample preparation and at atmospheric conditions. This non-destructive analytical technique will take you into a new dimension in microscopy.

- Intuitive easy to use software
- Rapid material identification using Raman spectral fingerprinting
- Absolute material distribution knowledge using chemical imaging
- No special sample preparation required
- Sub-micron spatial resolution for particulate analysis
- Rugged and portable design

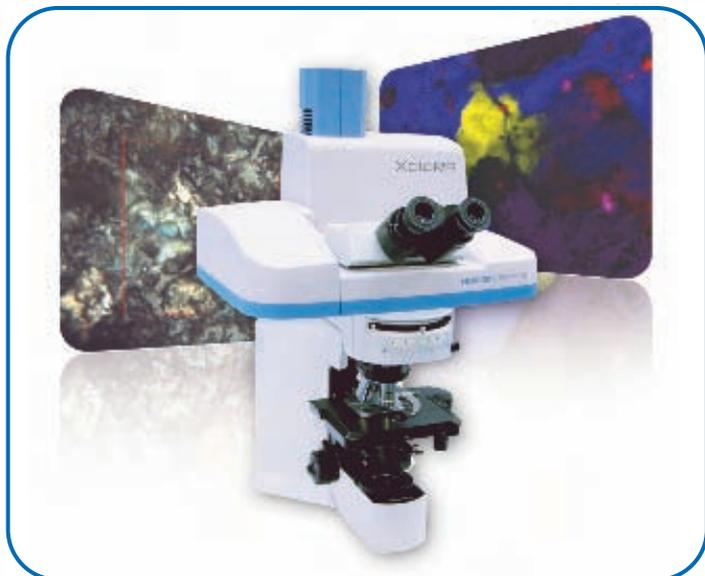


Figure 1: The XploRA™ Raman Microscope

### Intuitive operation

Intuitive operation through a new fully CFR compliant software modules including the Guided Operation wizard (GO!™), ensures complete ease of use and gets you up to full speed immediately. User defined templates or “analysis recipes” can be created/recalled at the touch of a button for those routine analyses or experiments. The Spectral ID database provides fast chemical identification of your sample components.

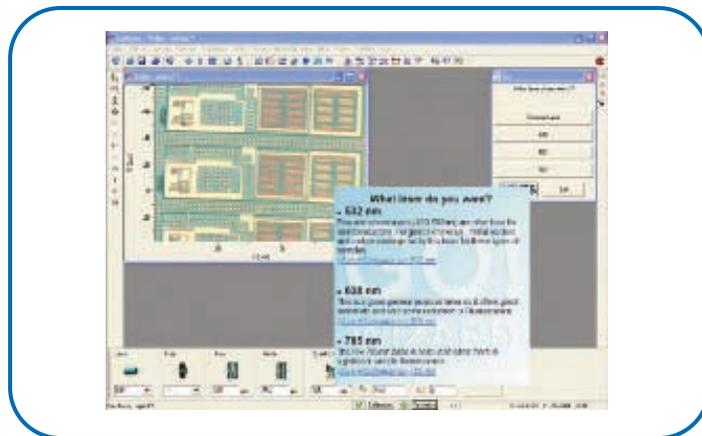


Figure 2: LabSpec+ software suite screenshot showing the GO! Wizard to ensure the best analysis conditions for your sample, even with little experience of Raman Spectroscopy!

### Smart Microscopy, why wait?

Visit our website to download the XploRA™ brochure and find out more about the system.

[www.smartmicroscopy.com](http://www.smartmicroscopy.com)

## DuoScan investigates CNTs

A. Zoubir, HORIBA Jobin Yvon S.A.S., Villeneuve d'Ascq, France

The unique DuoScan™ imaging system provides a way to record multiple laser excitations both for macro-mapping and sub-micron mapping.

In this study, we searched for isolated Single-Wall Carbon Nanotubes parallel to one another on a Silicon sample. The CNTs are about 100 nm in diameter, invisible by light microscopy. DuoScan™ provides a powerful method to locate these CNTs, and then to zoom in for a finer mapping analysis. Figure 1 shows a macro-scale image of the sample (area width > 1 mm) and the micro-scale image. This shows the superior spatial resolution achievable with a confocal microscope.

# Extending the range of the FluoroMax®-4 into the near-IR

Ray Kaminski & Stephen Cohen  
 HORIBA Jobin Yvon Inc., Edison, NJ, USA

The benchtop FluoroMax®-4 spectrofluorometer's standard Hamamatsu R928P photomultiplier tube has a sensitivity that rapidly drops at wavelengths > 800 nm. With the rise of nanoparticle and quantum-dot research, however, there is a growing interest in fluorescence measurements in the near-IR.

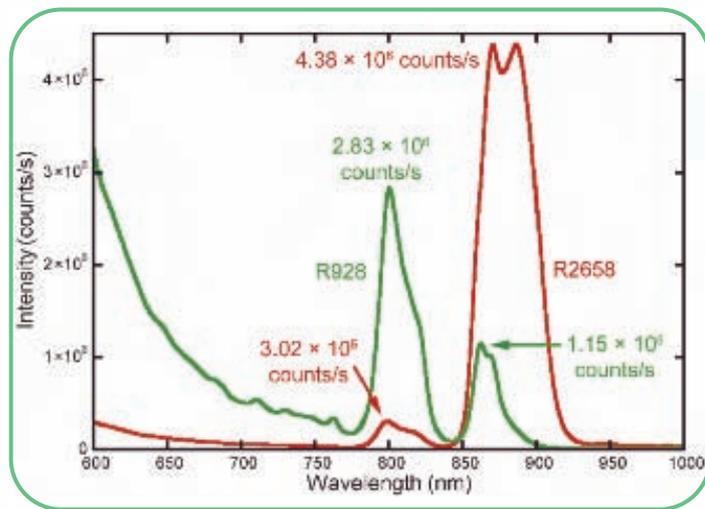


Figure 1: Comparison of spectra from laser glass taken with R928 (green line) and R2658 (red line) photomultiplier tubes in a FluoroMax®-4.  $\lambda_{exc} = 530$  nm; bandpass = 5 nm excitation and 8 nm emission; integration time = 0.1 s; 550 nm long-pass filter on emission; correction for dark noise.

The main problem with photomultiplier tubes at longer wavelengths is spontaneous emission from the photocathode, which increases the dark noise. Thus the R928P, for example, has a higher dark-noise background, than the Hamamatsu R1527—often used to demonstrate a high signal-to-noise ratio—though it is poor at detecting signals with wavelengths > 600 nm. Cooling the detectors lowers the dark noise, but also affects their range. For maximum dark-noise reduction, our Fluorolog® system is the obvious choice, because many exotic detectors can be adapted to this modular configuration. How, though, can we increase the sensitivity of the FluoroMax® in the near-IR? To answer this question, we modified the FluoroMax® to accept a Hamamatsu R2658P, with a range to just beyond 1000 nm, and compared it with the R928P.

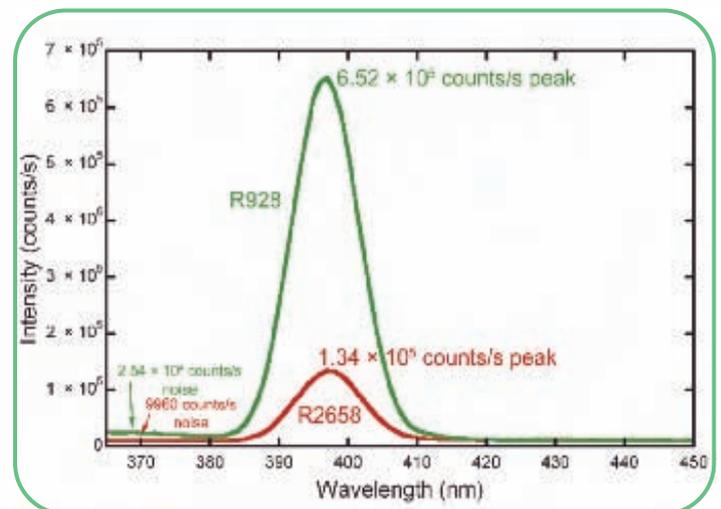


Figure 2: Water-Raman spectral comparison between R928 (green line) and R2658 (red line) photomultiplier tubes in a FluoroMax®-4. Integration time = 0.1 s;  $\lambda_{exc} = 350$  nm; all bandpasses = 5 nm; high voltage for R928 = 950 V; high voltage for R2658 = 1500 V.

Fig. 1 compares spectra of a laser-glass sample (normally used for IR calibration), run in a FluoroMax®-4 with a standard R928P and then an R2658P. The R928P dominates in sensitivity out to ~ 850 nm. Beyond 850 nm the R928P plummets steeply, while the R2658 is better. (By 900 nm, the quantum efficiency of the R928P is virtually zero.) The R2658 also gives surprisingly respectable performance throughout the visible and UV, as shown in a comparison of the standard sensitivity test of water-Raman spectra (Fig. 2) in a FluoroMax®-4.

If detecting the region from 850 nm to 1010 nm is important, then the R2658 is indispensable. A water-Raman spectrum from the FluoroMax® with the R2658 yields a peak with nearly 150 000 counts/s, which is still better than almost any other spectrofluorometer—except a standard FluoroMax® or Fluorolog®.

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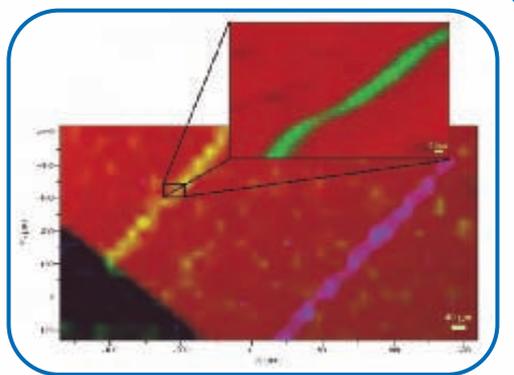


Figure 1: Isolated SWCNTs aligned on a Silicon sample. Courtesy of Dr Kalbac, Heyrovsky Institute, Prague, Czech Republic

# Antibody-Antigen Specific Interaction

B. Cherif<sup>1</sup>, A. Roget<sup>2</sup>, C.L. Villiers<sup>1</sup>, R. Calemczuk<sup>2</sup>, V. Leroy<sup>1,3</sup>, P.N. Marche<sup>1</sup>, T. Livache<sup>2</sup> and M.B. Villiers<sup>1,3</sup>

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<sup>2</sup>CREAB, UMR 5819 (CEA, CNRS, UJF), CEA Grenoble, France.

<sup>3</sup>Département d'Hépatogastroentérologie, CHU de Grenoble, Grenoble, France.

SPRi (Surface Plasmon Resonance imaging) technology is a powerful tool for the analysis and for determining antibody-antigen interactions.

In this experiment we demonstrate detection capabilities and quantify the interactions between several biomolecules. In our case we determine the kinetic curves of the interaction between, successively, mouse anti-hCG - hCG ; hCG - rabbit anti-hCG and rabbit anti-hCG - anti-rabbit IgG. Original surface chemistry combined with an electrochemical process allows the rapid coupling of biomolecules to the gold layer.

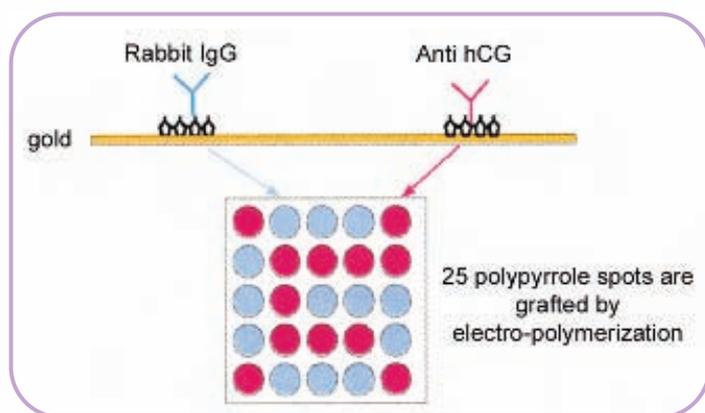


Figure 1: Two types of antibodies are first coupled to the pyrrole group and then grafted by electro-polymerisation onto the glass prism. Rabbit IgG and Anti-hCG antibodies from mice.

## 1) Interactions between rabbit IgG and anti-rabbit IgG from goat

Anti-IgG from rabbit are injected. Figure 2 represents the kinetics of interaction between the antibodies immobilized on the gold surface and the anti-rabbit IgG injected in the detection cell.

There is a clear interaction between the rabbit IgG (red curves) and injected antibodies. There is no reaction with other spots. The interaction between rabbit IgG and anti-rabbit IgG from rabbit is specific and can be monitored on a real time basis.

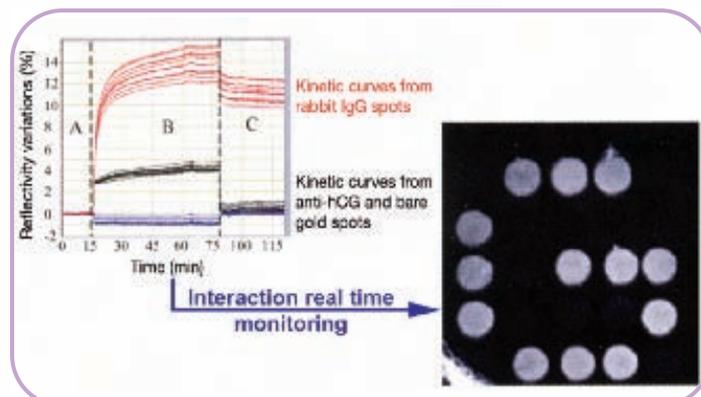


Figure 2: Reflectivity variations as a function of time following injection of anti-IgG from rabbit. A: PBS/Tween buffer; B: Injection of the anti-IgG; C: Rinsing with PBS/Tween buffer

## 2) Interactions between hCG - mouse anti-hCG, hCG - rabbit anti-hCG, rabbit anti-hCG - anti-rabbit IgG

A mouse anti-hCG antibody is firstly immobilised on the gold surface of the glass prism. The surface is then successively exposed to various solutions containing hCG, anti-hCG antibodies (different from those already immobilised), and a rabbit anti-IgG antibody (as described in the following figure).

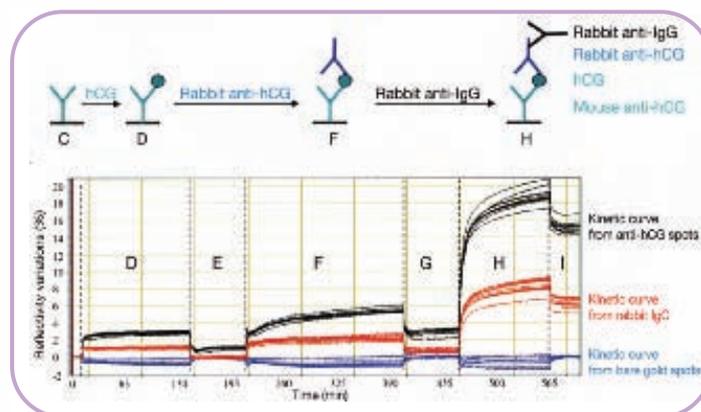


Figure 3: Kinetic curves on mouse anti-hCG spots, rabbit IgG and bare surface, following subsequent injections of hCG, rabbit anti-hCG and rabbit anti-IgG.

E, C, I: Injection of PBS 10 mM, Tween 0.05

D: Injection of 250 nM hCG

F: Injection of anti-hCG (monoclonal antibody 210 nM),

H: Injection of goat anti-rabbit antibody (polyclonal 220 nM)

## Conclusion

Our SPRi technology allows a real time study of biomolecular interactions. No additional labelling reagent is required, and a large number of spots can be analysed at the same time and in the same condition

Application note courtesy of Genoptics

# SLICE, A Database Approach to XRF Microscopy

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HORIBA was appointed exclusive distributor for EDXRF sales of the SLICE database and search software in 2007, and can now supply this powerful analytical tool in combination with the XGT-7000 – XRF microscope system.

Database software is well-developed in other spectroscopies, (such as FTIR, MS and Raman); however, until now has not been available in a robust form for EDS/XRF microscopy. The normal EDS result is a simple list of the elements present within a sample, with an estimate of their relative amounts, but with no final identification of the sample itself. For true material identification, this quantitative analysis is simply insufficient. SLICE, developed under an FBI contract with xk, Inc, is a database system capable of this true “identification” or sourcing of questioned materials. The FBI core requirements were for a database search facility with a fully relational x-ray database, with supporting metadata, comprehensive data mining abilities, and enhanced spectral display capabilities. The SLICE software meets all of these functional requirements to now give a complete sample identification from the elemental analysis, finally answering the question “What is this sample?”

Within SLICE, an EDS spectrum is treated as a signature, or compositional fingerprint of the source material, and is archived as the core component of the material record. In addition to the spectrum, qualitative and quantitative analyses of elements are included, as

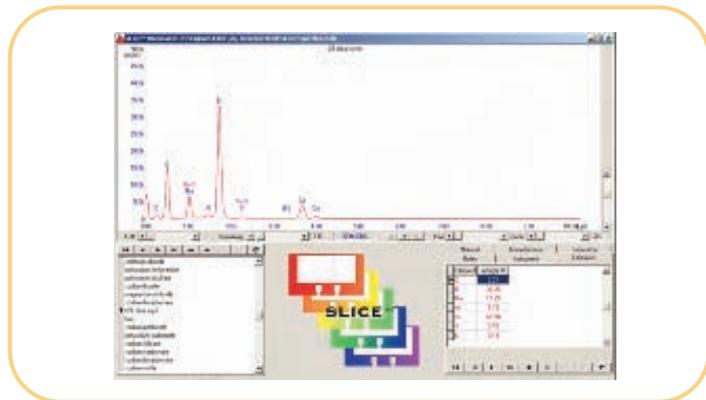


Figure 1: Main spectral display window

well as the supporting metadata. Provisions are made for the inclusion of text files and images, physical parameters, specimen preparation, manufacturer data, and more.

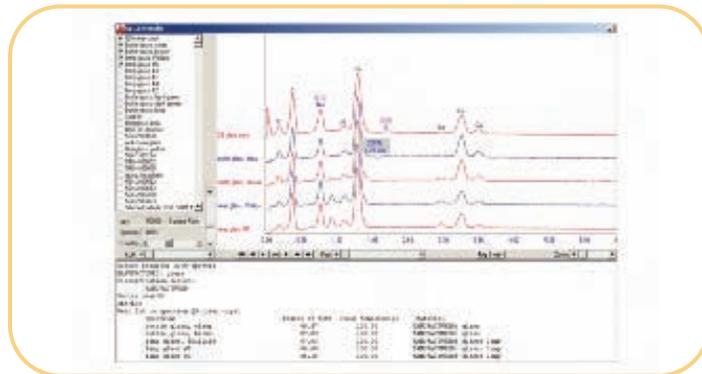


Figure 2: Search results window

Interrogation of the library is achieved by assessing the similarity of the target spectrum to those of the library members. Several manual and “automatic” methods are available to enhance and optimise searches. For instance, a “bracketing range” can be selected to optimise searching for peaks from elements which are considered important or diagnostic.

Each newly acquired spectrum is viewed in a Spectrum Display window within SLICE. The archival record is assembled within this window, including all metadata. The SLICE Explorer architecture enables powerful yet simple file management and spectral search and retrieval. A Search Results page displays spectra returned from the archive interrogation with any selected number of spectra overlaid for easy user interrogation. Spectrum separation and peak defined normalization functions provide clear views and discrimination of multiple spectra.

The example presented is a typical analysis using SLICE. The spectrum from an unknown “clear crystal” examined in an SEM is displayed in the Main Window (Figure 1). Usual EDS analysis is performed on the sample. The multiple Tabs (lower right corner of the display in Figure 1) are populated with information such as analysis parameters, administrative documentation, material description, etc. A Best Fit search is selected from the tools, and the search results are displayed (Figure 2). The spectra are ordered according to fit value, with the original spectrum at the top. From the list of ranked spectra, four were selected for display in this case. Note, EDXRF analysis would enable even more definitive identification from trace elements not detectable in SEM/EDS.

Regardless of whether the user relies on the community-developed database, or builds their own from materials of in-house relevance and origin, the analytical methodology embraced in the SLICE software provides the EDS community with a powerful enhancement of traditional microanalysis.

## HORIBA Jobin Yvon present at TERFS UK



*HORIBA Jobin Yvon booth at TERFS, UK*

On the 24th & 25th January 2008 in Teddington, UK, the National Physical Laboratory organised a conference on Tip Enhanced Raman and Fluorescence Spectroscopy (TERFS): Challenges and Opportunities.

This brought together scientists and instrument manufacturers to discuss the developments and future in the field of TERFS. The NanoRaman systems from HORIBA Jobin Yvon which combine Raman and AFM made our presence important and our team members found it very beneficial participating in discussions with attendees on the current situation of TERS.

## 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](mailto:mma-info@jobinyvon.com), or any of our international offices :

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<b>China:</b> +86 (0)10 8567 9966	<b>Other Countries:</b> +33 (0)1 64 54 13 00	

## FORTHCOMING EXHIBITIONS & CONFERENCES

2nd-7th March 2008

**PITTCON**  
New Orleans, LA, USA

1st - 4th April 2008

**ANALYTICA**  
Munich, Germany

6th - 10th April 2008

**ACS-Spring**  
New Orleans, LA, USA

7th - 11th April 2008

**Photonics Europe**  
Strasbourg, France

6th - 10th May 2008

**CLEO**  
San José, CA, USA

20th - 23rd May 2008

**HET Instruments**  
Jaarbeurs Utrecht, Netherlands

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

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