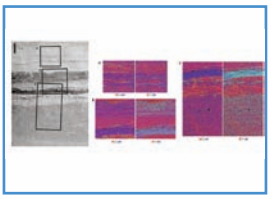
Molecular and Microanalysis Newsletter

Autumn 2009

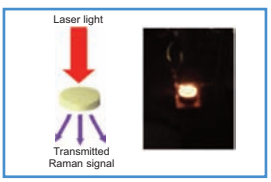
This newsletter is produced by HORIBA Scientific's Molecular & Microanalysis Team, to provide our customers, colleagues & friends with up-to-date information in the fields of Raman, fluorescence, SPRi & XRF Instrumentation and Applications.

What's inside:

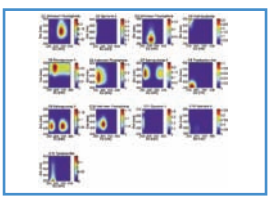
Page 2: Inferring Depositional Processes and Tempos in Fine-Grained Deep-Water Sediments by **X-ray Fluorescence** Mapping
Analysis of fine grained sediments using high spatial resolution **micro-XRF** has been used to understand settling, currents and debris flow in deep water settings.



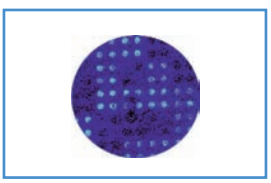
Page 3: Transmission **Raman** spectroscopy
Transmission **Raman** spectroscopy has been known since the very beginning of the Raman spectroscopy. However only recently has it been possible to develop this type of set-up for commercial application. It is the method which provides quick analysis of the average information about an entire sample.



Page 4: Using the **FluoroMax®** spectrofluorometer to determine water quality
Water quality is now globally recognized as one of the most important environmental concerns. Furthermore, fluorescence spectral analysis is also globally recognized as one of the most sensitive techniques for water quality characterization. Water travels through the environment carrying dissolved organic matter (DOM), made up of various chemical compounds, which have entered the water column from many sources.



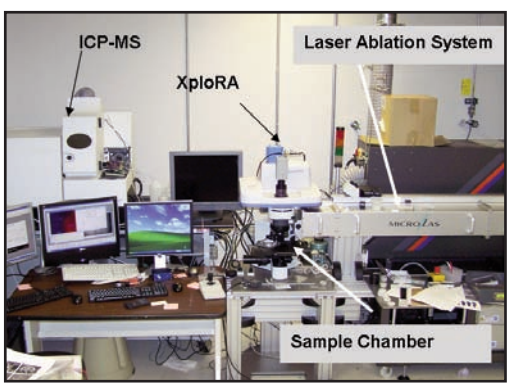
Page 5: Point Mutations of **DNA** Sequences
Genes can be mutated in several different ways. The simplest type of mutation involves a change in a single base along the sequence of a particular gene (much like a typographical error in a word that has been misspelled). In other instances, one or more bases may be added or deleted. Sometimes too, segments of a **DNA** molecule are accidentally repeated, deleted or moved.



Page 6: Our forthcoming conferences and shows

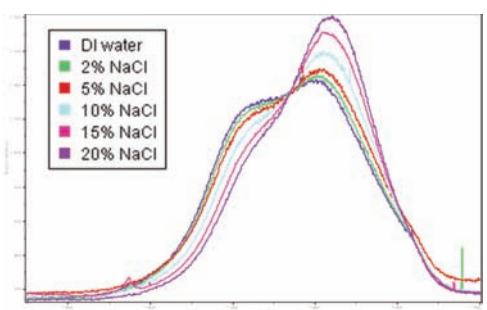
Novel Installation of Raman Microscope within a Laser Ablation Inductively Coupled Plasma Mass Spectrometer Platform Allows Geologists to Image Molecular and Atomic Information Simultaneously

Alan Koenig and Todor Todorov, United States Geological Survey, Denver, CO, USA
akoenig@usgs.gov



The United States Geological Survey has installed a Horiba Jobin Yvon XploRA Raman microscope platform in a novel integrated system within a Laser Ablation Inductively Coupled Plasma Mass Spectrometer (LA-ICP-MS) system at the LA-ICP-MS Facility in Denver, CO. The installation of the XploRA within the LA platform provides a unique opportunity to collect Raman information on a location of a sample destined for direct ablation for trace elemental analysis using the ICP-MS.

The Raman system provides critical information about geological materials including gas, liquid and salinity content within fluid inclusions, mineral polymorph differentiation and other structural variations within targets intended for trace element analyses by LA-ICP-MS. The composition of fluid and melt inclusions is used for the generation of ore deposit models to aid the mineral resource assessments. For fluid inclusions Raman spectroscopy is being used for measurement of salinity and detecting gas species such as CO_2, N_2, CH_4. In melt inclusions Raman spectroscopy provides information on quantity of water in the melt and can also be used for determination of the oxidation states of metals (Fe^{2+} vs. Fe^{3+}). By integrating these techniques in a single instrument we expect to simplify inclusion analysis, increase throughput and provide higher accuracy in the measurements.



Spectra showing the effect for sodium chloride on water structure – used for determination of salinity in fluid inclusions

The system is also intended for application development looking at spatial variations within tissues coupled to a recently developed cryogenically cooled LA sample chamber for trace element mapping of tissues.

Inferring Depositional Processes and Tempos in Fine-Grained Deep-Water Sediments by X-ray Fluorescence Mapping

Michael M. Tice, Texas A&M University: summarized by Yoshihiro Yokota, HORIBA Scientifc

Introduction

Fine-grained sediments are deposited in deep-water settings by hemipelagic and pelagic settling, contour currents, turbidity currents, and debris flows. These processes can be difficult to distinguish by their deposits in mud-dominated sequences. Since different modes of transport and deposition sort on grain “equivalence classes” based on various combinations of grain size, density, shape, and bed roughness, they are potentially distinguishable by characteristic patterns of grading imparted on silt-sized rutile and zircon grains. Moreover, suspension-load windblown sediment is characteristically enriched in zircons relative to other minerals, making Zr/Al or Zr/Ti ratios useful tracers for concentrations of windblown dust.

Core Samples

Cores were taken through rocks of the upper Permian Delaware Mountain Group, New Mexico. Samples displaying interbedded sandstone and shale, grading patterns, cross-laminations, and/or contacts between turbidities were selected for analysis. 25.6mm x 25.6mm square regions of slabbed core were mapped at 100µm resolution. All collection was performed using a 50kV tube voltage, 1mA current and complete vacuum in the sample chamber. The total collection time on each pixel was 0.5 s.

Results

Figure 1 shows a slab photograph with mapped areas indicated and paired false-color images of elemental maps. The left-hand image of each pair has Red = Si, Green = Zr, and Blue = Al. The right-hand image of each pair has Red = Si, Green = Ti, and Blue = Al. Pairings emphasize differential distributions of Zr and Ti. A) Cross-laminated very fine sandstone. B) From bottom to top: cross-laminated very fine sandstone, plane laminated very fine sandstone. C) From bottom to top: bioturbated silty mudstone, two cross-laminated very fine sandstone layers separated by a heavily bioturbated organic-rich mudstone, two heavily bioturbated mudstone layers, plane-laminated very fine sandstone.

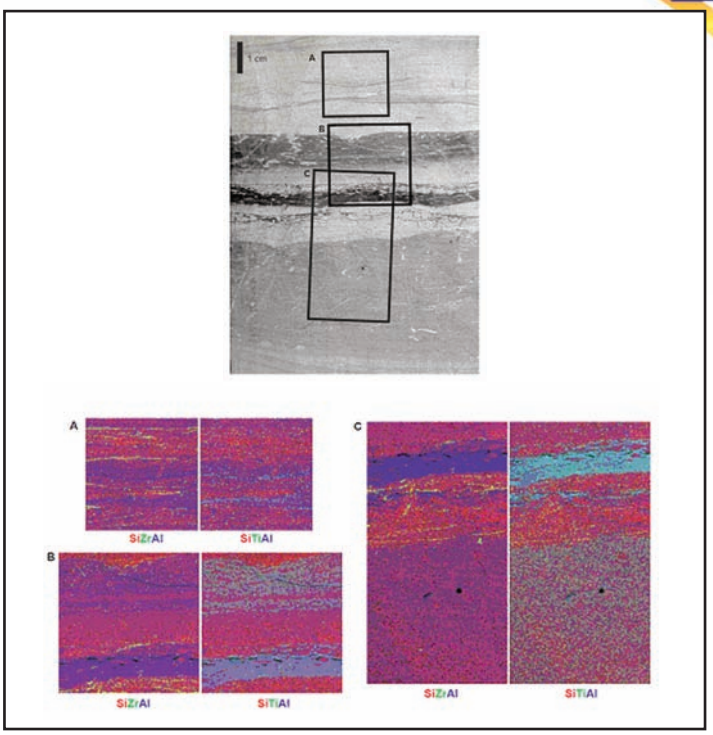


Figure 1

Conclusions

- In the rocks studied, Zr and Ti are present as fine mineral grains. They were sorted and deposited along with very fine sand (lower) to clay-size particles, with Zr present predominantly in the coarser fraction of this range and Ti in the finer fraction.
- Zr- and Ti-bearing grains are both concentrated in cross laminations. These layers are distinguishable from accumulations of windblown dust by their geometries and by the coupled distributions of Zr and Ti.
- Windblown dust accumulations are detectable as Zr-enriched layers or laminations.

Micro-Raman reveals sequence of non-int investigation

Dr T. Gal, J. Sandor, A. Karoly, Institute for Forensic Sciences, Dr Arna

When investigating fraud, counterfeiting, blackmail and anonym ment was altered after signing. In these cases, the crossing se When there are no intersecting lines between the printed text a cable.
During the printing process, micron-sized dry toner particles are characters. Incidently, thousands of discrete toner particles ran A new method based on confocal Micro-Raman spectroscopy features on documents even if there are no intersecting lines. M document was printed after signing. This method is non destruc

For more information, please see our full Application Note avail **http://www.horiba.com/scientific/products/raman-spectrosc**

Transmission Raman probes volume

Dr Renata Lewandowska, HORIBA Scientific
Dr Arnaud Zoubir, HORIBA Scientific

Transmission Raman spectroscopy is based on the collection of Raman light propagating along the direction of the excitation laser through a sample. Unlike its back-scattering counterpart, vastly predominant in most dispersive Raman setups, the transmission (or forward) configuration allows one to analyze the full volume through which light travels (not only the surface).

Although the Transmission Raman principle has been known since the early days of Raman Spectroscopy, it is only recently that commercial instrumentation has become available to work in such configuration. HORIBA Scientific is now offering this new technique for fast pharmaceutical assays and tablet screening, based on fibered systems wich are robust and easy-to-use.

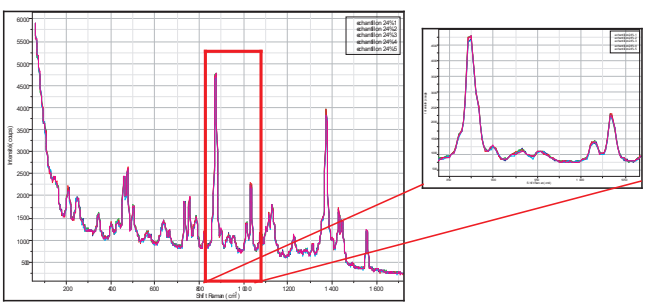
Although a pharmaceutical tablet is not transparent, laser light is able to penetrate and propagate thanks to the light scattering effect occurring in the whole volume of the sample. The light coming out on the other side contains Raman signal which holds the chemical information of the whole tablet, giving access to its chemical profile in a matter of seconds:

- API concentration
- Polymorphs analysis
- Crystallinity

As in classical Raman, Transmission Raman is non-contact, non invasive and requires no sample preparation. Moreover, the measurement is insensitive to particle-size effects or sample inhomogeneity or orientation.

Although initially of interest for pharmaceutical samples, it can be extended to polymers, biological tissues, or other even non-transparent samples where volume analysis is required.

A pharmaceutical tablet Illuminated by the laser light



Spectra of a sequence of 5 measurements recorded on a pharmaceutical tablet including propranolol as the API. The sample has been taken off and put back between the 2 measurements demonstrative the ruggedness and reproducibility of the transmission probing method.

Sample courtesy from Jonas Johansson, Astra Zeneca.

ersecting lines for forged document

ud Zoubir, HORIBA Scientific

nous letter cases, forensic experts frequently have to find out whether a docu-
quence of ink lines often needs to be determined.
and the pen ink lines, standard techniques used for sequencing are not appli-

e melted and flattened by pressure on the surface of the document to form the
domly contaminate the surface of the paper.
allows to investigate the chronological sequence of printed and hand-written
leasuring the toner ink spatter spectrum over the signature ink reveals that the
ctive and requires no sample preparation.

lable at
copy/application-notes/forensics/

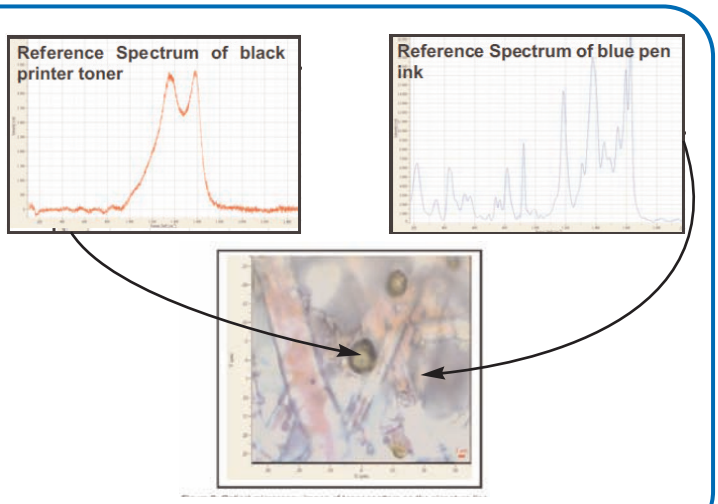


Fig 1: Optical microscopy image of toner spatters on the signature line

Using the FluoroMax® spectrofluorometer to determine water quality

Dr Adam Gilmore, HORIBA Scientific

Introduction

Water quality is now globally recognized as one of the most important environmental concerns. Furthermore, fluorescence spectral analysis is also globally recognized as one of the most sensitive techniques for water quality characterization. Water travels through the environment carrying dissolved organic matter (DOM), made up of various chemical compounds, which have entered the water column from many sources. The amount of DOM in water and its chemical composition vary in space and time. Light's interaction with DOM is a function of its chemical make-up; thus fluorescence spectroscopy can provide information about the amount and type of DOM in a water sample. Because the chemical composition of DOM is determined by its original source material and the processing occurring in the environments through which it travels, DOM characterization using fluorescence spectroscopy can help find the source of DOM, as well as the pathway the water transporting it has followed.

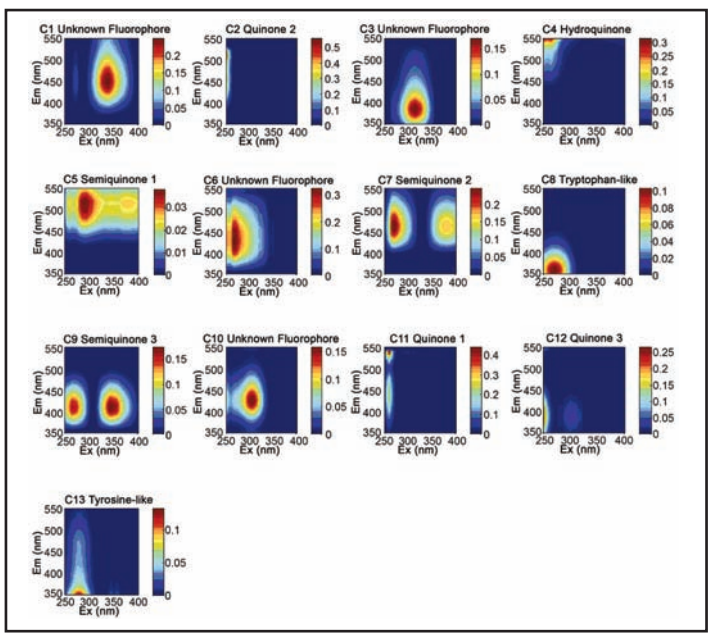


Fig.1: PARAFAC analysis on an EEM from a water sample. Thirteen separate modeled components describe 98% of the variability.

The amount and type of DOM in local water systems is important for urban planning, ecological studies, and understanding effects of trace amounts of organic compounds on living organisms. Fluorescence spectroscopy, especially the excitation-emission matrix (EEM), is becoming more popular for determining the amount of DOM contamination in water. Using EEMs to determine polyaromatic hydrocarbon and "humic substances" (soil-derived compounds) seems especially effective. Brian Bergamaschi and co-workers at the U.S. Geological Survey have been recording EEMs of water sources in California to monitor water quality.

Result

Water samples were quickly filtered in the field through 0.2 µm pre-combusted glass-fiber filters, chilled, and shipped overnight to the U.S.G.S. laboratory in Sacramento, California. After the sample equilibrated to 25°C, it was placed in a 1-cm^2 quartz cuvette inside the sample compartment. An EEM was constructed by measuring a sample's fluorescence via a FluoroMax® spectrofluorometer at thousands of combinations of excitation (200–400 nm) and emission (220–600 nm) wavelengths. An automatic shutter protected the samples from photobleaching. While recording fluorescence, the signal was corrected with the reference detector.

Such EEMs can be decomposed into excitation-emission components, representative of fluorophores, via statistical methods. Bergamaschi, *et al.*, used parallel factor analysis (PARAFAC) to decompose data into separate components, and provide values proportional to each component's signal. Fig. 1 is an example of PARAFAC decomposing an EEM. With separate components determined, plots of loading (proportional to signal) versus excitation and emission can be drawn (Fig. 2.).

Ecological and geological studies by means of parallel factor analysis of EEMs have been shown to be a useful application involving HORIBA Scientific spectrofluorometers.

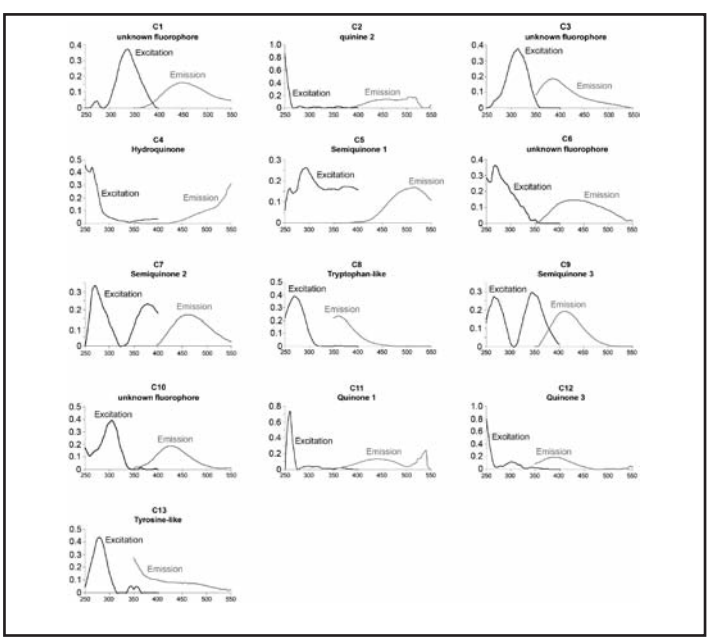


Fig.2: Loading (y-axis) vs. wavelength (x-axis) for the components in Fig. 1.

Point Mutations of DNA Sequences

Michaël Canva - Laboratoire Charles Fabry, Institut d'Optique, CNRS, Sophie Bellon, GenOptics, Chiraz Frydman, Horiba Scientific

Introduction

Genes can be mutated in several different ways. The simplest type of mutation involves a change in a single base along the sequence of a particular gene (much like a typographical error in a word that has been misspelled). In other instances, one or more bases may be added or deleted. Sometimes too, segments of a DNA molecule are accidentally repeated, deleted or moved.
DNA mutations generate genetic disorders that are responsible for hereditary diseases and have a predominant role in many diseases such as cancers.
GenOptics SPRi sensitive technology (Surface Plasmon Resonance imaging) is a method developed to detect DNA point mutations.

Experiment

The gold sensor surface (the glass prism) was covered with a mixed self-assembled multilayer based on electrostatic interactions made of 3 layers of MUA: 11 Mercapto-undecanoic acid, PEI: poly(ethyleimine) and ExtrAvidin. Matrices composed of 100 or 196 spots (10 x 10 or 14 x 14 matrices) were prepared and data from all parallel hybridisation kinetics was collected.

As this technique is highly sensitive and very precise it enables accurate identification of mutations even at a single base mismatch in an oligonucleotide sequence.

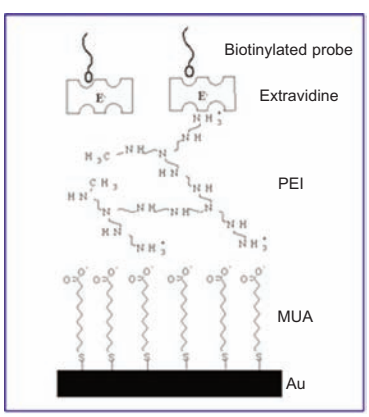


Fig. 1: A mixed self-assembled multilayer

In addition, the recognition specificity of functionalized spots is such that each spot reacts only with the relevant complementary sequence within a mixture of different sequences. For example, by reconstituting patients genotypes with appropriate oligonucleotide mixtures (homo or heterozygote), it is possible to determine their genotype with no ambiguity.

Results

The system makes it possible to monitor simultaneously several hundred measurements, and additionally, directly compare the effects induced by the variation of one or several parameters. An example is given in Figure 2, showing the variations of an hybridisation signal between probe and target of various oligonucleotide lengths.

Only one gene microtiter plate was prepared with different genotypic sequences grouped in pairs of various length presenting pairwise mutations (either by mutation or deletion).

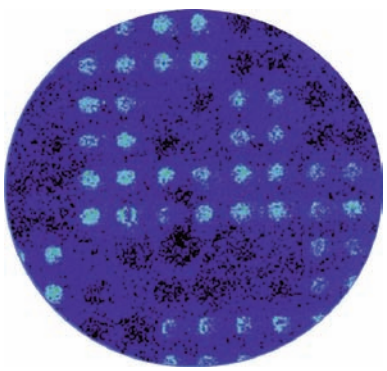


Fig. 2: Image of the gold surface bearing 100 spots distributed on an area of 16 mm², having reacted with an oligonucleotide mixture reproducing the conditions of a patient genotype.

A mixture of target oligonucleotides including one of the two (wild or mutated) species, corresponding to each of the pairs was injected in the detection cell.

Using this technique, it is possible to quantify in a single run all kinetics corresponding to an entire column of the sensor matrix, and this for each sequence studied.

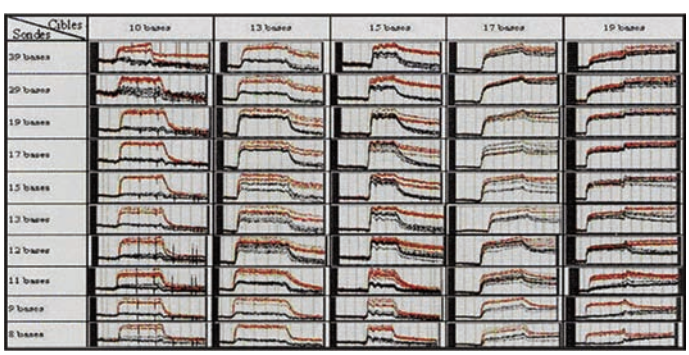


Fig. 3: Hybridisation kinetics recorded during 20 minutes periods. Rows show targets of various lengths

Dr. Ewa Hartwell is pronounced winner of XploRA prize draw by HORIBA Scientific

Dr Izhar Ul-Haq , HORIBA Scientific

To promote the launch of the new XploRA Raman microscope, HORIBA Scientific UK distributed leaflets highlighting the excellent features and performance of the instrument. A link at the back of the leaflet allowed all prospective customers to register for a prize draw for a state-of-the-art satellite navigation system. The prize draw took place on the 13th of January and the lucky winner was announced as Dr. Ewa Hartwell of Cambridge Biostability Ltd., Cambridge, UK. The image shows Dr Izhar Ul-Haq presenting the prize.

FORTHCOMING EXHIBITIONS

6-10 September
Diamond
Athens, Greece

14-17 September
ICXOM20 Conference
Karlsruhe, Germany

21-25 September
EUPVSEC
Hamburg, Germany

21-25 September
Lacona VIII
Sibiu, Roumania

6-8 October
Opto
Paris, France

18-23 October
ECASIA 09
Antalya, Turkay

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

www.horiba.com/scientific

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