



RAMAN MICROSCOPES FROM HORIBA SCIENTIFIC

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Horiba Scientific Company, the division of the Horiba group of companies, is the world-known manufacturer of measuring and analytical equipment. Horiba Company was founded in 1945 by Masao Horiba, the student of physics at the Kyoto University. The first products of the company were pH meters, their production started in 1950. Today, Horiba company group is a large international concern including 42 companies of more than 15 countries. Horiba produces a wide range of measuring and analytical instruments for research and development centers and laboratories, health care institutions and clinics, environmental monitoring, industrial monitoring, semiconductor industry, automobile industry.

Horiba Scientific Company is the world leader in Raman scattering spectroscopy (Raman spectroscopy). Jobin Yvon and Dilor, the French companies which were included into Horiba group some time ago, stood at the origins of the Raman equipment; they design and produce Raman spectrometers for over 40 years. They have developed such new items as the world's first commercial Raman microscope and the world's first remote fiber optic probe.

ZAO "Nytek Instruments", which was founded in 2003, is the main distributor of Horiba Scientific Company in Russia and in EEU countries. It is one of the main dealers of Raman spectrometers, spectrofluorimeters, glow discharge spectrometers and other spectral equipment. The company has been twice acknowledged as the best Horiba Scientific dealer in Russia. "Nytek Instruments" has many other partner companies such as AIST-NT (scanning probe microscopes), Rigaku Raman (portable spectrometers), EuroVectorf. Among the customers of "Nytek Instruments" there are many leading industrial and scientific organizations in Russia, such as Rosatom, Norilsk Nickel, "Polyus" gold mining company, Moscow and St. Petersburg State Universities, National Research Institute for Optical and Physical Measurements, several research centers of Russian Academy of Sciences.

At the, the ZAO "Nytek Instruments" organized the workshop within the Analitika Expo exhibition which took place in April 2015 in Moscow. The latest samples of Raman microscopes produced by the Horiba Scientific corporation were presented there.

Raman spectroscopy (Raman scattering spectroscopy) is non-destructive, non-contact, fast method of physical and chemical analysis, which does not require special sample preparation. It is possible to analyze samples in solid, liquid, or gas states, as well as, powders, aqueous solutions, suspended solids, suspensions, etc. It could even be possible to analyze packed production without opening the package, since polyethylene and other transparent polymers, as well as many glasses, have very weak Raman cross section. Measuring process does not require vacuum or any other special conditions. Spectra are normally registered at atmospheric pressure and room temperature. If necessary, it is possible to work in a wide temperature and pressure range, or under controlled atmosphere, since modern spectrometers are supplied with a wide choice of additional options and modules for such tasks.

The phenomenon of combination all light scattering was theoretically predicted by Adolf Smekal in 1923, shortly after discovery of the Compton Effect. Smekal suggested that a similar phenomenon of



the photon scattering with frequency shift can take place not only for X-rays, but for optical wavelengths as well. The combinational scattering effect was experimentally discovered in 1928, by two groups of researchers independently and almost at the same time. One group was from Moscow, leading by Mandelstam and Landsberg, and another was from Calcutta, a group of Indian scientists Raman and Krishnan. In Russia and USSR the effect was so-called "combinational light scattering", which was originally proposed by Mandelstam, whereas in the Western literature it is traditionally referred to as Raman effect or Raman scattering, and optical devices built on this principle are named Raman spectrometers.

The physical nature of the Raman scattering phenomenon is as follows. A photon contacting with sample can be absorbed by a molecule that resides in the proper quantum state with a definite energy. As a result of such absorption, the molecule passes into the virtual state, and then, after emitting the photon, it passes again into its proper state, which could be different from the original state. In such a case, the emitted photon has different energy than the initial photon. This difference is equal to the gap between the final and the initial levels of energy. It can be positive (Stokes shift) or negative (anti-Stokes shift). The Stokes shift is statistically more probable, since it happens more often that the absorbing molecule is initially in its ground (lower) state.

As we can see, the process of Raman scattering is inelastic, unlike the common elastic (Rayleigh) scattering, when the photon does not change its energy. It is noteworthy that since Raman scattering requires both photon absorption and emission, its probability is much smaller than that of Rayleigh scattering, so the registration of Raman spectra needs detectors of much higher sensitivity.

The key feature of the combinational scattering effect, which allowed it to be a powerful spectral technique, is the fact that energy difference between the absorbed and emitted photons (or frequency shift that is the same in terms of the quantum theory) is exactly equal to the transition energy between the molecule's different states. Therefore, the pattern of such shifts is unique for each particular chemical compound, representing some kind of "passport" or "fingerprint" of the molecule.

Raman shifts recorded by the spectrometer, normally have a dimension of wave numbers, and

are usually expressed in reciprocal centimeters (cm^{-1}). The spectra provide both qualitative and quantitative information. The position of peaks indicates the nature of a chemical compound and of its functional groups; the intensity indicates the concentration; the line width serves as an indicator of structural disorder of environment. The peak position shift upon temperature or pressure changing could also provide quite important information.

Raman spectroscopy together with infrared (IR) spectroscopy forms the method group named vibrational spectroscopy. Both types of spectroscopy study the same vibration levels of molecules, but in different ways: IR-spectrometers register absorption (or emission) while Raman spectrometers - inelastic light scattering. Both methods complement each other. Being used together, they could provide complementary structural information about complex molecules containing various functional groups. Raman spectra, but not infrared ones, show intense signals of nonpolar functional groups, double and triple carbon bonds, symmetric vibrations of aromatic rings. On the contrary, vibrations of polar substitutes, in particular, hydroxyl, carbonyl and amine groups are more easily seen in IR-spectra. Note also that unlike the conventional Fourier techniques, the classic dispersion scheme with diffraction grating and multichannel spectral detector are generally used in IR-spectroscopy and Raman devices, that determines a number of advantages of Raman scattering spectroscopy (they will be discussed further).

The laser is normally used as a photon source for Raman spectrometer. Since Raman scattering cross section is inversely related to the fourth power of the wavelength, and silicon CCD with the red border of the operating range of ~ 900 nm are currently the most effective multi-channel detectors for spectrometer, it is optimal to use lasers from the near UV band to the near infrared band (200-800 nm). The most commonly used lasers are operating at visible wavelengths: 633 nm He-Ne, 514 nm Ar, 473 nm solid-state, etc. More rarely, a 1064 nm Nd:YAG laser is used. Lasers with longer wavelength are practically not used in Raman spectroscopy.

It is rather important for practical applications that many various lasers could be connected to the spectrometer. Usage of lasers of near IR band enables removing of disturbing fluorescence signals from the spectrum. On the other hand, dependence of Raman scattering process on the cross section



of wavelength for some compounds and functional groups has sharp resonance peaks in the blue and UV range, and selection of a laser with the wavelength close to the resonance is optimal for such samples.

The modern professional Raman spectrometers are usually combined with optical microscopes, most often with the confocal ones. Such devices are named Raman microscopes or microspectrometers. They have a number of important advantages. Since the exciting laser in such device is focused in a small area near the focus of the microscope objective with the size of the order of the emission wavelength, Raman spectral signal is from the same area. Thereby, we provide measurement localization with the possibility of spectra binding to different points of a sample surface. Moreover, the use of confocal scheme with sufficiently small aperture provides three-dimensional localization of spectrum measuring. An optically transparent volumetric sample can be examined by layers, wherein each layer of the sample (with the thickness about 1 micron) can be studied separately, without destroying the sample and without making cross sections as such.

Microspectrometers are usually equipped with high-precision motorized scanning tables to perform two-dimensional (three-dimensional) pointwise spectra recording from a specified area (volume) of sample. Such procedure is also called Raman mapping.

It is also important to note that the entire laser light flux is focused in a small pre-focal area, so the strong objective of microscope collects the major part of scattered light, and even a small laser power (~ 1 mW) is sufficient for efficient operation of a device. On the other side, the three-dimensional spatial filtering in the microscope due to confocal diaphragm (aperture) enables completely to get rid of the entrance slit in the device, and thereby to circumvent the long-standing problem of optical spectroscopy – the need for compromise between luminosity of a device and its resolution.

As we said before, the silicon CCD array detectors are most commonly used for Raman spectra registration. Modern technologies provide efficient cooling of the detector array without refrigerants (such as liquid nitrogen), using a vacuum insulation and the Peltier cooler. This reduces the dark current almost to zero level, so the fast high-quality detector electronics has a quite low readout noise. Therefore, if previously just the detector's noise (thermal noise and noise of electronics) was the determinative

noise, when the spectrum is recording, then currently the random noise of incoming light flux (shot noise) described by the Poisson distribution has become determinative and in no way related to the detector operation. The relative level of shot noise is inversely related to the square root of absorbed photons number, and if the experiment parameters (initiating laser power, exposure time, etc.) are correctly chosen, it could be minimized to a negligible level. Noteworthy, since some years ago the CCD detectors have a function of photon induced current multiplication (EM-CCD), which makes possible to register each single absorbed photon, ensuring a rapid spectra recording even at small scattering cross section.

Coming back to the comparison of Fourier IR and Raman dispersion spectroscopy, it is possible to say that all common disadvantages of dispersion scheme relatively to the Fourier scheme (slow operation speed, low luminosity, low signal/noise ratio) in Raman microspectrometers were successfully overcome and, moreover, the dispersion Raman scattering spectrometers have several important advantages over the Fourier IR-devices. Low-power lasers can be used for excitation; therefore it is no more risk of sample heating or destruction, as well as appearance of disturbing signals of thermal (Planck) emission. Being used together with confocal microscopy, Raman scattering spectroscopy provides much better spatial resolution (~ 1 micron versus 20-30 microns of good Fourier IR-microscopes) and the possibility of three-dimensional mapping. The atmosphere, particularly water vapors, does not affect the spectra quality anymore, so the sample compartment draining or evacuation, which represents a big problem for IR spectroscopists, is no longer required. The spectra of aqueous solutions, including those of organic compounds, can be recorded without any undesirable absorbance of spectral lines by the aqueous medium.

Confocal Raman microspectrometers provide a wide range of applications of Raman scattering spectroscopy method in various fields of science and technology. It is possible to obtain a chemical image of tablets and medicaments surface in pharmaceuticals, i.e. to visualize the components distribution, and it is possible to perform depth profiling and optical sectioning as well. It is possible to perform the express analysis of quality and authenticity of raw materials, including through the transparent packaging without opening. The polymers synthesis and monomers conversion can

be observed in real time by the spectral signal in the chemical laboratory. (Spectrometers are equipped with special remote fiber optic probes for insertion directly into the reaction mixture). Mapping of stresses and strains of silicon layers, as well as the study of impurities and contaminants distribution can be performed in the semiconductor industry. Three-dimensional spectral scanning, e.g. of cells, including in living environment, as well as lipids, proteins, amino acids studying can be done, using the inverted optical microscope. Due to submicron resolution of modern confocal microscopes, Raman mapping is widely used in nanotechnologies; it can be used for spectra analysis of nanotubes, graphene sheets and other nanomaterials. The fast non-destructive analysis of chemical composition of a sample can be performed in criminalistics. Finally, Raman spectrometers find themselves in such unusual field as archeology and art history. They are used for exhibits authenticity determination, artifacts origin detection, artistic pigments and precious stones identification on large samples. Portable Raman spectrometers can be easily taken along for measurements directly in archaeological excavations or in museums.

When choosing a Raman spectrometer, one should pay attention to its several key features. The most important of them are parameters of exciting laser (lasers), such as wavelength, aperture, beam divergence, line stability. The advantages of using different lasers were discussed before. Other quite important features of a Raman spectrometer are including the spectral range, spectral resolution, spectrograph design (quality of the input aperture display, aberration absence, multipath propagation suppression, spectrum scanning linearity), spatial resolution (for microspectrometers), signal decay, minimum and maximum values of measured Raman shift (both Stokes and anti-Stokes), quality of spectral detectors, availability of automation equipment, configuration flexibility and availability of additional options or modules for specific applications (heating or cooling, measurements in liquid or gas environment or in the presence of magnetic fields, etc.), possibilities of working with polarized exciting light, connecting of probes allowing remote or in situ analyses.

Availability of the lower limit of the measured shift is inherent feature of any Raman device related to the fact that elastically scattered (i.e. without frequency changing) photons of the exciting emission must be as fully as possible removed from

the spectrum of scattered light, otherwise their flux will inevitably get through a series of multipath propagation inside the spectrograph and will flash the detector array; and it will be simply impossible to see weak Raman signal on this background. Unfortunately, there is no way to physically remove the strictly defined wavelength from the spectrum - only just some band of finite width, here is a reason of discussed limitations. At the same time for some Raman scattering experiments, measurement of just small Raman shifts (or, in other words, the low frequencies in the spectrum) is very important. Therefore, the minimum value of measured shift, the opportunity to approach close to the excitation wavelength is a very important characteristic of Raman spectrometer.

There are two common ways of blocking of the initial exciting wavelength: holographic cutoff filters for specific wavelength or additional double monochromator operating in the dispersion subtraction mode. The second method is technically much more difficult, since it requires two additional cascades of monochromator precisely synchronized mechanisms for diffraction gratings rotation, aberrations compensation in the spectrograph's optics and accurate knives installation of the intermediate slit. However, it still remains the only solution providing Raman shifts measurement that are maximum close to the excitation line at any excitation wavelengths, from UV to IR, including tunable lasers using. And currently, T64000 device of HORIBA Scientific Company is a unique commercially available Raman device, assembled under such scheme.



T64000



Horiba T64000 instrument implements three successive cascades of the monochromator. First two of them can work consistently in dispersion subtraction mode, providing filtering the excitation line, and the ability to measure both the Stokes and anti-Stokes shifts of 3 cm^{-1} . The monochromator optics and special non-planar diffraction gratings with curved grooves installed on synchronized sine mechanisms provide almost complete compensation of aberrations and precise reflection of the input aperture (confocal diaphragms) on the intermediate slit and then on the output plane of device. The device is completely achromatic over all operating spectral range from UV to near IR. In terms of features T64000 device is the unique solution for the most difficult research problems in Raman spectroscopy.

T64000 device, despite all its advantages, could be regarded as rather expensive and complicated in operation. So, it makes sense to pay attention to more simple optical schemes based on the shutoff holographic filters. Such devices are currently offered by many manufacturing companies, including, certainly, HORIBA Scientific Company – well-known LabRAM and Xplora brands are in its range. Let us consider what is essential in the design of such device and in its selection for solving one or another experimental problem.

The cutoff filter can either block all wavelengths smaller than the specified, in this case it is called the edge filter; such filters are used to study the Stokes shifts. Or it may remove a small band in the spectrum close to the specified wavelength, then it is called the notch filter; such filters allow studying both Stokes and anti-Stokes shifts. In both cases the filter has a finite transition band that just specifies the minimum shift that can be observed, when it is applied. For edge filters this value (half-width of the transition band) is typically about 50 cm^{-1} and for notch filters – about 100 cm^{-1} . Recently special shutoff notch filters appeared on the market for visible wavelengths, made on the base of the technology of volumetric Bragg gratings (VBC). For them, the half-width of the band is only 5 cm^{-1} ! However, they are still available only for a limited set of wavelengths in the visible range. It is important, however, to understand that when using a cutoff filter for optimization (width reduction) of their transient response, their accurate tuning is very important by the angle of the input beam incidence. This is especially critical for VBC filters, even with small error of position they simply stop working. It is also evident that for each wavelength of the laser its own cutoff filter

is required, with which the device is supposed to work; and when changing the excitation laser, the filter needs to be replaced (and tuned by angle). We emphasize that the problem does not consist in the filter acquisition for desired wavelength – the filters are now available practically for any laser suitable for Raman scattering experiments; the problem consists in its installation / replacement and position tuning. It is optimal for an experimenter when these operations will take place with minimal human intervention, in the best way – fully automatic. Therefore, we have formulated an important requirement for Raman device: automatic change (switch) of cutoff filters with automatic tuning of their angular position.

This functionality is present in both above stated types of HORIBA Scientific devices: LabRAM and Xplora. Moreover, the possibility to switch and tune the above described VBC filters is realized in LabRAM device that makes this spectrometer the most versatile solution for complex tasks of Raman scattering spectroscopy, and it is very easy to use.

Let us consider LabRAM device in more detail. Its basic optical scheme was first time proposed about 15 years ago, and since then it did not changed significantly. One internal excitation laser (633 nm, He-Ne) is built in the device, but any other external laser could also be connected through the optical port without any restrictions. All optical connections in the device are made directly, without any optical fibers. This enables exciting laser polarization control as well as scattered light polarization measurement.

The device monochromator is built on the basis of asymmetric Czerny-Turner option using aspheric (toroidal) mirrors that provide the absolute minimum (of commercially available tools) level of multipath propagation and detector false glare, as well as the complete absence of chromatic aberrations. The usage of non-planar compensating gratings enables complete elimination of spatial aberrations in the first two orders and provision of excellent display quality of small input aperture on the output plane of the detector – within 40 microns, i.e. less than 2 pixels by width on the standard CCD array. Note that a user can easily replace gratings during the device operation, depending on the experiment conditions; HORIBA Scientific Company offers the wide range of high-quality gratings produced by the company. Also, LabRAM device has the record high scanning range linearity, the error does not exceed 0.5 cm^{-1} .

Due to high quality imaging and long focal length of spectrograph (800 mm), LabRAM device has the record high spectral resolution: 0.2 cm^{-1} at a

wavelength of 633 nm with the grating of 2400 grooves per one millimeter. It is important to note that the spectral resolution of this device besides the focal length depends on many other parameters: when the spectral resolution determining, the wavelength of laser excitation and grating parameters should be at least specified. High spectral resolution is required for closely spaced lines differentiation, analysis of line-shape change, small spectral shifts definition with pressure and temperature changing. The value of spectral resolution is one of the most important characteristics of Raman spectrometer

Olympus optical microscope (direct or optionally inverted) of research grade is integrated in LabRAM device and the input optics unit with controlled confocal diaphragm is integrated as well. Therefore, LabRAM device is a fully-featured confocal Raman microspectrometer. The spatial resolution of LabRAM device corresponds to limiting formulas of theoretical optics, in particular at the wavelength of 488 nm with the lens 100X / NA = 0.9 the lateral resolution does not exceed 0.5 microns, and the axial does not exceed 1.5 microns (the spatial resolution is always determined at specific operating wavelength and particular lens of a microscope). It should be noted that HORIBA Scientific Company is the sole major manufacturer of Raman microscopes on the world market that offers such devices with complete three-dimensional confocal spatial filtering – LabRAM and Xplora

The modern version of LabRAM device called LabRAM HR Evolution was originally introduced by the manufacturer in 2012 and immediately won the prestigious international Best New Scientific Instruments Award. The key features that distinguish the new version from the previous implementation of this device are: improved optics quality, full automation, large number of new add-on modules for measurements in special conditions, possibility of quick Raman mapping, and the ability to work with VBG filters for low-frequency transitions observation which we mentioned above.

Optical range of LabRAM HR Evo is extended to the record range in the industry from 200 to 2200 nm. Two switching independent optical channels are realized in the device's input cascade: the lens channel optimized for minimum losses in the visible spectral range, and the image channel, completely achromatic for operation in UV and IR.

LabRAM HR Evo automatics includes lasers (internal and external) switching control, the incident power control (by means of neutral filter), cutoff filters control, polarizers and analyzers control,



LabRAM Evolution

confocal diaphragm control, precision auto-tuning means of optical path and auto-calibration means (to reduce drifts), motorized sample table control.

Up to three optical ports for spectral detectors can be installed in LabRAM HR Evo device, and HORIBA Scientific Company offers its customers the wide variety of detectors: from standard CCD to fast EM-CCD, the near-infrared detectors based on InGaS, PbS, etc., various types of photomultiplier tubes (PMT).

The manufacturer of LabRAM HR Evo device offers the huge range of various additional options and modules, in particular: various types of thermo-cells, high pressure cell, cryostats, magnets, optical tweezer, protective cover, means for macro-spectrum measurements of a large area (volume) sample, special remote optical heads and remote fiber optic probes for remote measurements and measurement "in-situ", various options of motorized tables for 2D and 3D mapping.

In general, one can say that the flexibility level in terms of configuration for user's tasks, on the level of automation and technical features, LabRAM HR Evo device is the absolute market leader among the systems of this class, and for more than 15 years (including the time period of the previous version) is the most popular Raman scattering spectrometer in the world, acquired for scientific laboratories.

HORIBA Scientific Company has presented its Xplora device, first produced in 2010, as a compact and easy-to-learn instrument, convenient both for solving scientific problems of the entry level, and for an analytical laboratory as well. The device is currently available in two versions: Xplora Plus



Xplora Plus

(based on Olympus BX direct microscope) and Xplora Inv (based on Nikon Ti inverted microscope). This device is also a complete confocal microspectrometer equipped with motorized mapping means and providing adequate spatial resolution.

Three solid lasers (wavelength 532 nm, 638 nm, 785 nm) are installed in Xplora device, and they are integrated into the device case to minimize the optical path. Optical connections are implemented without optical fiber, and the possibility of polarization control is available.

The monochromator of Xplora device is also assembled on the basis of Czerny-Turner scheme with quality input aperture imaging. The focal length of monochromator is small (200 mm), but the turret is mounted inside with 4 gratings with different groove densities. The appropriate grating selection provides controlled spectral resolution within 1.5 cm^{-1} in the whole operating range of wavenumbers ($50 - 3500 \text{ cm}^{-1}$).

Xplora device is small in size (not more than $50 \times 50 \text{ cm}$ for Xplora Plus option), it does not require special conditions for installation and operation. The device's interface is user-friendly even for an unskilled user. The device is fully automated: switching of lasers, filters, polarizers, gratings and other elements is performed by the software.

Xplora Inv device keeps the full functionality of Nikon Ti high-end inverted microscope, and that allows performing of sample's simultaneous optical and spectroscopic studies, the fluorescence spectra measuring, high aperture immersion lenses using. The manufacturer positions this device as the

optimal solution for biological and biochemical laboratories

LabRAM HR Evo and Xplora Plus/Inv devices are controlled by modern HORIBA LabSpec software. The current (6th) version of this software has a number of significant advantages. The multi-user interface with security keys and different levels of access to the device's settings, the ability to control the entire device's automation, built-in visualization tools (including 3D), macro facilities, spectrum analysis means, advanced spectral data processing means, their exports and imports in various formats, reporting facilities are implemented in this software. The program kernel supports multi-threaded processing and provides coping with very large 4-dimensional data arrays in three-dimensional Raman mapping. The user-friendly flexible interface provides easy learning of device operation even by non-specialists. The module for small particles spectra analysis (useful for operation with optical tweezer), the multivariant analysis module, the spectrum identification module, as well as database packages of Raman spectra of different chemical compounds in accordance with the user request may be installed. In addition to the software basic functionalities.

It should be said separately about software tools for 2D and 3D Raman mapping via motorized table. The special SWIFT feature is implemented in LabSpec 6 (both for LabRAM and for Xplora), which provides high-speed multi-faceted strobe data reading from multichannel detector in synchronization with the table movement without its stop.

This function is especially useful when working with EM-CCD fast detector, as it enables the full use of its speed performance and hold time reduction (the sample's specified point passage) to 1 ms.

To summarize, we would like to emphasize that HORIBA Scientific Company currently offers to its customers a wide range of Raman spectrometers for wide variety of tasks, from fundamental research to routine analysis, that meet the highest requirements, as well as the richest possibilities for devices configuration according to user's requirements and a varied selection of additional modules and components for special measuring instruments. ZAO "Nytek Instruments", the general partner of Horiba Scientific Company, is always pleased to offer highly professional service, consultations of qualified specialists, technical and methodological support, training, and internship opportunities in Horiba laboratories.

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