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Advanced 3-D confocal microscope for Raman imaging spectroscopy.

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Abstract A spectral confocal microscope, "*Nanofinder* ®*fh*, especially designed for simultaneous high spatial resolution and sensitivity is presented. Multiple set of operation modes as well as options and sophisticated 3D-software make this device an universal and perspective tool for wide range of material analysis at scales well below 1 μm.

1. Introduction

A global tendency of technical science is a micro- and nano-technology. Submicron- and nanosize objects with different molecular or crystal structure are subject for investigations. Among many different methods of submicron resolution material analysis Raman spectroscopy has some undoubting advantages:
-nondestructive remote optical control;
-do not demands any special sample preparations;
-high spatial resolution capability with tightly focused laser spots;
-high selectivity and clear material finger printing recognition.

These evident advantages have been recognized by both researches and producers of commercial equipment for microstructure material analysis and high performance Raman microscope systems are now available on the market [1].

These devices are mainly concentrated on spectroscopy application. Those, using moving stages for Raman intensity mapping, can propose spatial resolution about 1 μm lateral and 2 μm axial. However, modern developments in nanotechnology request higher special resolution and selectivity (thing film analysis, semiconductor industry, 3D optical memory) without any degradation in sensitivity.

The way of resolution improvement in optical microscopy is a confocal technique. For Raman intensity mapping (imaging) it is a very natural system built-up principle, because of possibility of using of relatively low power lasers (1-100 mW) – focused in submicron spots they have enough intensity to generate Raman scattered signals from submicron samples with reasonable S/N ratio. In confocal microscopy a spatial resolution is determined by pinhole size. In practice the pinhole diameter is always a trade-off between resolution and signal to noise (S/N) ratio. A detailed analysis of influence of pinhole diameter on both of these parameters was done in [2]. Typically pinhole diameter is equal to diameter of projected Airy disk image onto the plane of pinhole location (Airy intensity distribution is generated on a sample surface when plane optical wave is focused by microscope objective lens). In such a case an optical slice thickness, corresponding to a full width at half maximum (FWHM) of the intensity distribution, generated by point-like object behind the pinhole is given by:

$$A = \sqrt{\left(\frac{0.88\cdot\lambda_{em}}{n-\sqrt{n^2-NA^2}}\right)^2+\left(\frac{\sqrt{2}\cdot n\cdot PH}{NA}\right)^2}$$

where λ_{em} is emission wavelength, n is refractive index of immersion liquid, PH – object side pinhole diameter, NA – numerical aperture of microscope objective lens.

Lateral resolution is given by:

$$L = \frac{0.51 \cdot \lambda_{exc}}{NA}$$

where λ_{exc} is an excitation laser wavelength.

With λ_{exc} =488 nm, λ_{em} =500 nm, NA=0.95 (air) or 1.4 (oil) and n=1 (air) or 1.5 (immersion oil) the values of optical slice thickness A and lateral resolution L are correspondently 1.13 μm and 0.26 μm (air); 0.9 μm and 0.18 μm (oil).

In this paper we describe a versatile spectral confocal microscope "*Nanofinder*®*fh*, which has being designed for Raman imaging has spatial resolution close to highest, achieved by laser confocal microscopy.

2. System layout

In this chapter principles of design and features of optics of "*Nanofinder*®*fh* are discussed. Possible options are listed.

The spectral microscope was developed by Tokyo Instruments, Inc. with financial support from Japan Science and Technology Corporation. The main targets for the system design were:

-high spatial resolution;
-high optical throughput of both illumination and detection channels;
-high sensitivity of signal detection equipment;
-high mechanical and thermal system stability;
-flexibility and ability of simple upgrade by multiple optional equipment;
-fully motorized and computer controlled;
-universal software for both: gathering and presentation of 1-, 2- and 3-D data for spatial, spectral and temporal imaging.

Now we have developed an universal system, which can be combined with both inverted and up-right type microscopes, CW and pulsed lasers, TE cooled CCD and photon counting PMT or APD detectors. Different scanners (piezo-stages, galvanic mirrors or step motors) can move sample or laser beam for subsequent reconstruction of 3D-confocal images of object of interest. Fixed changeable or permanently variable pinhole configurations can be selected for optimal system performance in every specific application. UV (from 244 nm), VIS (390-1100 nm), and IR (up to 1300 nm) models are available now for Raman or fluorescence imaging spectroscopy.

Combination of confocal spectral microscope with scanning probe microscope (SPM) is realized. This option means an installation of SPM onto a XY piezo-stage. Such combination can simultaneously supply topographic and spectroscopic information from transparent samples. A noble metal covered SPM probe has already demonstrated its subdiffraction Raman image resolution with using SERS (surface enhances Raman scattering) technology [3,4]. It permits in principle to increase lateral resolution of the system up to 50 nm with simultaneous sensitivity increasing. SNOM (scanning near field optical microscope) fiber as a light source for optical imaging below diffraction limit can also be used.

Operation of 3D confocal microscope in both reflection and transmission modes is realized with developed transmission illumination option. This option may be used for SRS (stimulated Raman Spectroscopy) imaging applications [5].

The basic system layout with piezo-stage scanner and joined illumination-detection pinhole is shown in Fig.1. Irradiation from CW or pulsed laser can be delivered to "***Nanofinder ®***" through single mode fiber or by system of mirrors (in the case of UV or femtosecond laser sources). Motorized beam expander (4,5) with adjustable output divergence and possibility to install inside it a pinhole (for spatial filtration of lower quality laser sources) sets exit beam parameters for effective filling of entrance aperture of used microscope objective lens. From compromise considerations of high optical throughput and smallest focused spot size an illumination laser beam should have diameter (at $1/e^2$ level) equal to entrance aperture of microscope objective lens (29).

Depending on application it is possible to select an appropriate geometry for illumination-detection channels separation. As a splitting element (9) a holographic beamsplitter, notch-filter, edge-filter or partly reflection mirrors can be used. All of these optical components have different features, which should be taken into account in the process of system configuration. For example, holographic beam splitters with sharp edge and narrow gate for laser line suppression and flat transmission curve can produce autofluorescence under powerful laser irradiation, which increases straight light in detection channel and decreases system sensitivity. Edge filters with multiple dielectric coating, in opposite, do not produce meaningful fluorescence but have modulated transmission curve that can provide some difficulties in broadband spectra interpretation. As additional spectral filters (12) both these elements can be used successfully. In critical applications when both low straight light level and uniform transmission curve are required a metal partly reflection mirrors can be applied. These both advantages have cost of laser illuminating power losses.

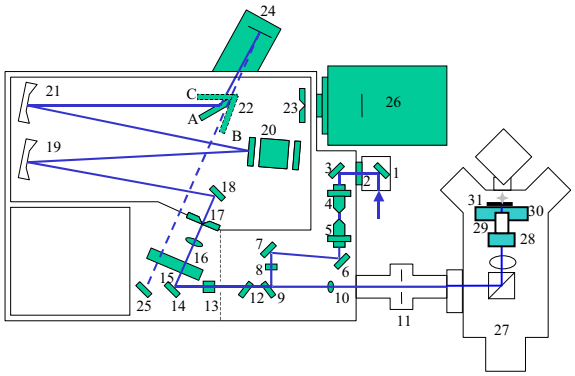

Fig.1
Optical layout of "*Nanofinder ®*".
1-entrance mirror or fiber collimator; 2-plasma line filter; 3, 6, 7, 14, 18, 19, 21, 22, 25–mirrors; 4,5–beam expander; 8–half wave retardation plate; 9-beamsplitter; 10, 16–objective lenses; 11–pinhole; 12-additional filter; 13–Glan-Taylor prism; 15-filter wheel; 17,23-spectrometer slits; 20-turret with gratings; 24-CCD; 26-PMT; 27-microscope; 28-Z piezo-scanner; 29-objective lens, 30-XY piezo-stage, 31-sample.

Pinholes size and location are key point of a confocal microscope. We put position of motorized settled individually preadjusted pinholes in imaging plane of microscope, optically conjugated with sample. In this position a single pinhole (11) plays two roles: works as illumination and as a detection pinhole. This principle dramatically simplifies procedure of illumination- detection pinhole system alignment. Simple maximization of laser power passed through pinhole (illumination) automatically maximizes signal, passed through the same pinhole (detection) in detection channel. Since laser beam has high spatial quality (or it already passed through a single mode optical fiber or additional filtrating pinhole) the main pinhole size can be selected for high transmission of illumination beam (70-90%). Pinhole's size is mainly determined by demands of detection channel. As it was told before, an optimal pinhole size should be equal to diameter of projected Airy disk image onto the plane of pinhole location for used objective lens. Airy disk diameter for uniform illumination of microscope objective lens is given by:

$$D_A = \frac{1.22 \cdot \lambda_{exc}}{NA}.$$

For objective lens 100X with NA 0.95 (1.4 for oil immersion lenses) it is 0.63 μm (0.43 μm). In imaging plane of microscope it becomes 63 μm (43 μm). This is an optimal pinhole size for confocal low signal detection. If signal is strong enough it is possible to decrease pinhole size, going to improving in spatial XYZ resolution up to 1.4 times (by cost of decreasing throughput in illumination-detection channels).

Mapping in described basic system is realized by moving microscope objective lens in axial direction by piezo-scanner. XY-mapping is realized by moving sample, installed with vacuum chuck on the top of the piezo-stage. In the cases, when sample should be enclosed inside heavy cryostat, oven or flowing cell a galvanic mirror scanner exchanges XY piezo-stage, exchanging sample scanning by laser beam scanning method.

Raman or luminescence signal, scattered by sample, passes through additional filter set for suppression of laser line and Glan-Taylor prism (polarization measurement) and focuses onto entrance slit of spectrometer. This is 0.56 m imaging spectrometer with 4 changeable gratings and 2 exit ports (detection by PMT or CCD). For weak, but broadband fluorescence one grating is exchanged by metal mirror. In this case full signal with high efficiency can be gathered onto photocathode of PMT.

Switching mirrors (14) and (22) in the detection channel can be configured for "Direct Image" mode. In this mode laser beam is automatically defocused on the sample surface and magnified Raman or luminescence image of sample is projected onto cooled to –90°C scientific grade slow scan CCD.

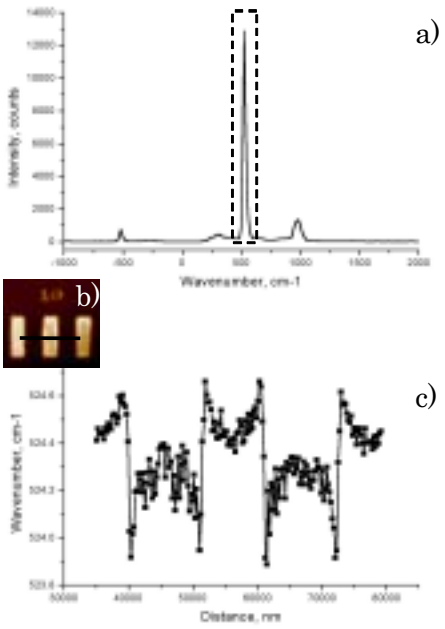

Fig.2

a) Raman spectrum from Si. Stocks line at 520 cm^{-1} is used for mapping. Dashed box selects spectral window from full Raman specter from CCD.
b) Raman line intensity mapping of SiO_2/Si structure. Average value of signal in selected spectral window is used for mapping image.
c) Raman shift cross-section (along black line). Raman line shift corresponds to stress on the surface.

3. Performance examples

A principal concept of system operation is illustrated in Fig.2. Raman or luminescence line maximum value, average intensity in selected spectral window, wavenumber, corresponding to signal maximum or center of gravity of spectral peak – are 4 different functions witch can be

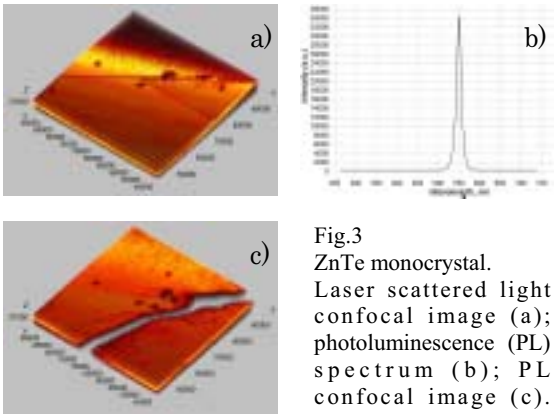

Fig.3
ZnTe monocrystal.
Laser scattered light confocal image (a); photoluminescence (PL) spectrum (b); PL confocal image (c).

mapped simultaneously using system software. Up to 20 different spectral windows can be selected for single mapping process. So, up to 80 different pictures, corresponding to different spectral windows and different functions within selected windows can be received after single mapping. Additional fifth function is a saving full spectrum in every mapping point.

In compare with usual reflective image, spectral imaging can brings more clear information about sample nonhomogeneity, as shown in Fig.3.

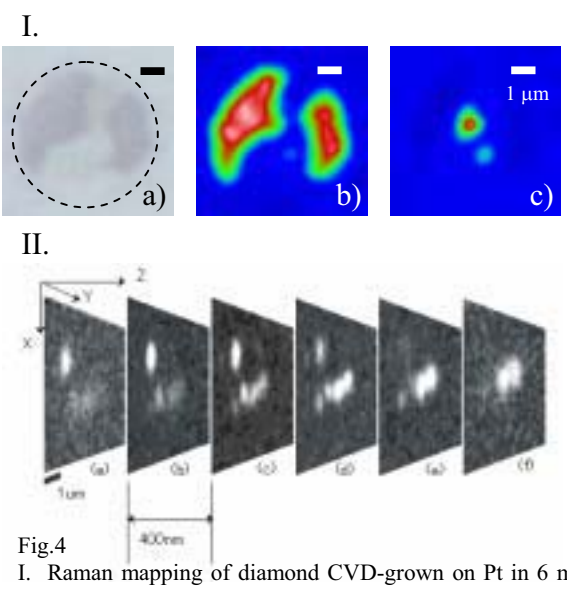

Fig.4
I. Raman mapping of diamond CVD-grown on Pt in 6 mm diameter hole (shown by dashed circle) in SiO_2. Scale bar 1 µm.
a) Optical reflection image of the deposition pit. The darkest regions are those where Pt was removed and a Si substrate became exposed.
b) Mapping was carried out at 520 cm^{-1} (Si Raman line).
c) Map recorded at 1332 cm^{-1} (diamond line).
II. Vertical slicing of diamond grains (mapping was carried out at 1332 cm^{-1}). The images (a-f) each separated by 0.4 µm. Scale bar, 1 µm.

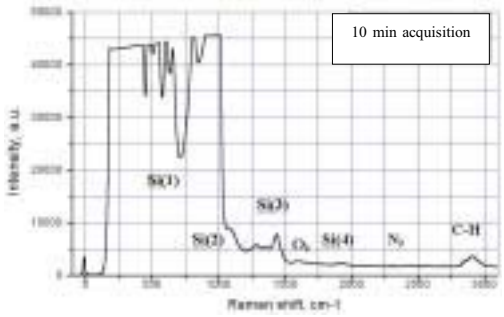

Fig.5
Raman spectrum from Si wafer. Sensitivity test for Raman confocal microscope. Si(4) peak is clear detectable. The first and the second orders of Si Raman specter (Fig.2(a)) are in deep saturation. Laser 488 nm, power 8mW. Grating 300 l/mm.

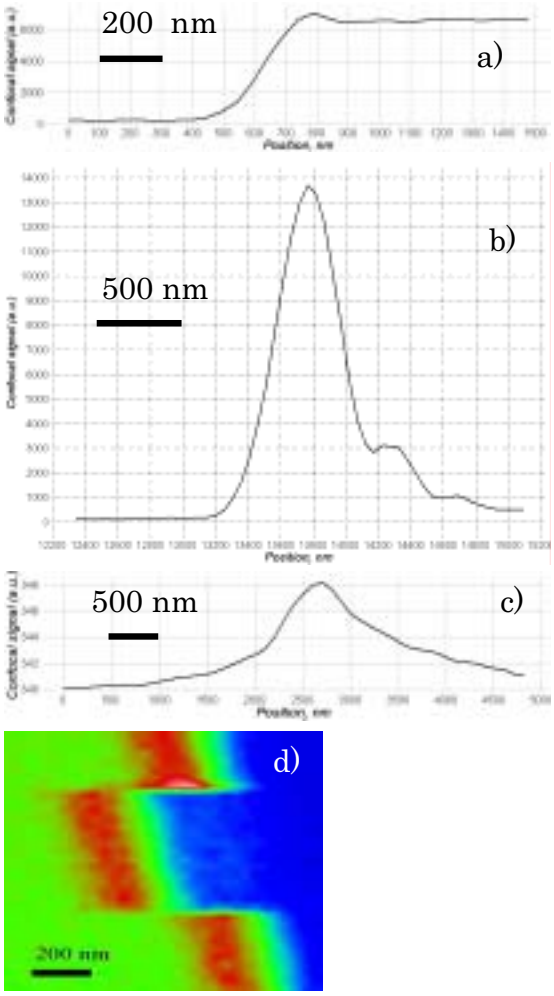

Fig.6
Spatial resolution of "*Nanofinder®fh*.
a) Lateral sharp edge response. Signal drops from 90% to 10% for 200 nm. λ=514 nm, objective 100Xoil, pinhole 60 µm.
b) Z-profile with objective lens moving along optical axis of microscope. Reflection from mirror. FWHM 500 nm. λ=514 nm, objective 100Xoil, pinhole 60 µm.
c) Z-profile fluorescence from dye molecular monolayer on glass substrate. λ_{exc} 488 nm, λ_{em} 520 nm , objective 100X, pinhole 60 µm.
d) Raman image at 520 cm^{-1} of Si wafer edge. λ_{exc} 488 nm, objective 100X, pinhole 60 µm.

Simultaneous 2 spectral windows scanning for Raman imaging of chemical vapor deposition (CVD) grown diamond array is presented in Fig.4 [6]. Locations of deposition of different materials are clearly distinguished. Slicing in depth with step 400 nm for the similar sample allows obtaining sectioning of the diamond deposition in axial direction and shows high axial optical slicing ability.

High sensitivity of the Raman confocal microscope is demonstrated in Fig.5. The possibility to detect forth order of Si Raman specter may be considered as a sensitivity test for Raman detection system.

High spatial resolution of the system can be confirmed by Z-scanning profiles and XY-edge response functions (Fig.6). In the case of scattered laser light detection from mirror (Fig.6,b) Z-response has FWHM less then 500 nm. With the center of gravity criteria it provides autofocusing accuracy better then 5 nm RMS. Optical slicing of molecular monolayer with FWHM about 1 μm is presented in Fig.6,c.

System XY edge response in scattered laser light (distance of confocal signal drop down from 90% to 10%) is about 200 nm (Fig.6,a). The similar lateral resolution with Si Raman detection is demonstrated by Fig.6,d.

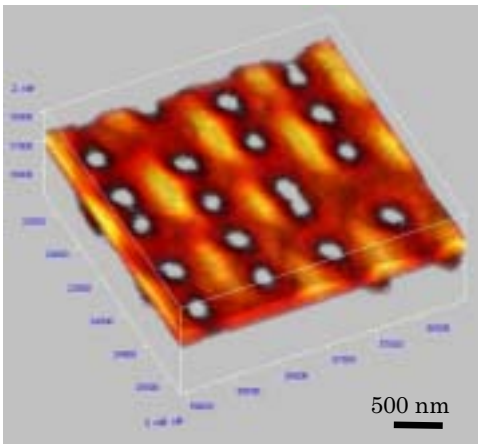

Fig.7
3D image of DVD surface in "Laser Confocal Microscope " mode. Scale bar 500 nm.

Fig.7 gives example of DVD confocal image in "Laser confocal microscope mode".

4. Conclusion

A versatile spectral confocal microscope "*Nanofinder* ® *f'h*is presented. Principles of the system built-up and functionality are described. High spatial resolution (axial (mirror surface)<500 nm, optical slicing~1 μm; lateral~200 nm) and sensitivity (4-th Si Raman peak detectivity) are demonstrated. Sophisticated multifunctional 3D software ensures data acquisition as well as visualization and processing. The "*Nanofinder* ® *f'h* can be an universal and easy upgraded spectral confocal microscope for a wide range of material investigations at nano- and micrometer scales.

References

1. Renishaw "Ramascope", Jobin Yvon – Horiba "LabRamInfinity", Nicolet "Almega™ Dispersive Raman", Chromex "Senturion™ Raman Microscope", Kaiser Optical Systems "HoloLab Series 5000 Microscope, WiTec "CRM 200" etc.

2. S.Wilhelm, B.Grobler, M.Gluch, H.Heinz. Confocal Laser Scanning Microscopy. Principles. – Microscopy from Carl Zeiss, microspecial 40-617e/02.00.

3. Raoul M.Stockle, Yung Doug Suh, Volker Deckert, Renato Zenobi. Nanoscale chemical analysis by tip-enhanced Raman spectroscopy. – Chemical Physics Letters 318 (2000) 131-136.

4. Norihiko Hayazawa, Yasushi Inoue, Zouheir Sekkat, Satoshi Kawata. Near-field Raman scattering enhanced by a metallized tip. – Chemical Physics Letters 335 (2001) 369-374.

5. Hiro-o Hamaguchi. Nonlinear Raman Spectroscopy. – Nonlinear Spectroscopy for Molecular Structure Determination. Prepared under the auspices of the IUPAC Commission on Molecular Structure and Spectroscopy, ISBN 0 632 04217 6.
6. I.Kudryashov, A.Gvozdev, V.Zhizhimontov, A.Volkov, E.Vanagas, S.Juodkazis, H.Misawa. Three-dimensional mapping of photoluminescence and Raman spectra. Proc. 25th Int. Conf. Phys. Semicond., Osaka 2000 (Eds. N.Miura and T.Ando), pp.1751-1752.