Application of Spectral Technique in the Scanning Probe Microscopy: SFOM (Scanning Fluorescence Optical Microscope)

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We report the technical characteristics and principle scheme of the SFOM (Scanning Fluorescence Optical Microscope) "Nanofinder". This device is produced jointly by NT-MDT Co. (Russia) and Tokyo Instruments Inc. (Japan) and allows using confocal optics to get Raman and photoluminescence spectra in every XYZ point of the sample with spatial resolution up to 200 nm. The possible applications are fluorescence and lifetime analysis of compounds, contaminants, defects, and stress in semiconductors, films, liquid crystals, biological salines, etc.

The scope of the SPM application involves almost all parts of the modern science. However, the common disadvantage of such systems is impossibility to get information about chemical structure of the sample and passing fast and ultrafast processes that is the subject of spectroscopy.

In recent years modern optical spectroscopy has made great progress associated with achievements in laser technique of ultra short pulses, incredibly increasing sensitivity of multi channel optical detectors, production of unique holographic spectrum elements and widespread fibers for gathering light and also intensive using of computers for real-time image processing. At the same time obtaining the local spectrum characteristics along the surface is a very attractive purpose. Scanning luminescence and Raman spectroscopy mapping is a very powerful instrument for material analysis in different fields of science and industry. This technique can be used for investigations of various materials (solid, liquid, biological samples) and provides information about structural and compositional properties; it is sensitive to chemical elements distributions on the surface and inside the samples. ICCD-based spectroscopy system can provide spectral information in less than 1 sec. Usually there is very low light signal level in spectroscopy; therefore decreasing the analyzing surface (but increasing the spatial resolution) drastically diminishes the incoming signal and one has

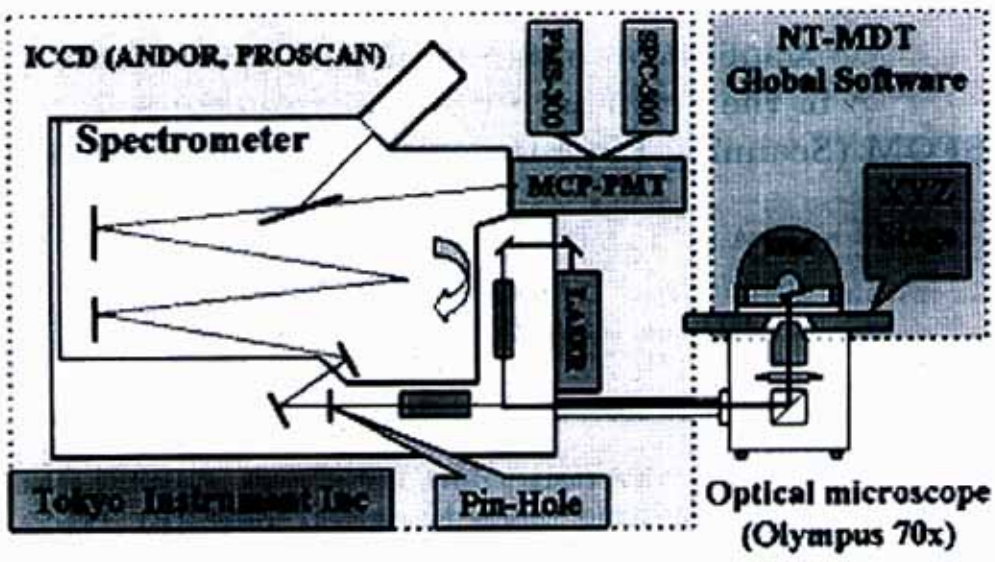

Figure 1. Nanofinder. Optical scheme of the SFOM.

to use cooled detectors and increase the exposure time to several minutes. The whole system must have small heat drift and high stability. As for the scanning, then the most famous producer of such devices is the German firm PI. It develops scanners with the range up to 150 μm and with the feedback founded on capacitance and gauge sensors. In this case the important problem is synchronization of the scanning process with the process of getting signal from the optical detector.

One of the evident devices in this scope is the RM series Raman microscopes of the UK firm Renishaw. For instance, the system RM1000 has spatial resolution of ~ 1 μm and spectral resolution of ~ 1 cm^{-1}.

In this article we consider the characteristics and the principle of operation of the Scanning Fluorescence Optical Microscope (SFOM). This project called Nanofinder was developed by NanoTechnology-MDT Co. jointly with the Japanese firm Tokyo Instruments for getting spectrum images with the spatial resolution of one hundred nanometers. Several types of detectors (PMT or MCP/PMT, cooled CCD or cooled ICCD, GPS) allow to carry out fluorescence and lifetime analysis of compounds, contaminants, defects, and stress in semiconductors, films, liquid crystals, biological salines. The principle optical scheme of the SFOM is shown in Figure 1. The system consists of two global parts: the scanning and the spectrum ones plus the combining software. Both XY (for the sample displacement up to 100 μm) and Z (for micro objective displacement up

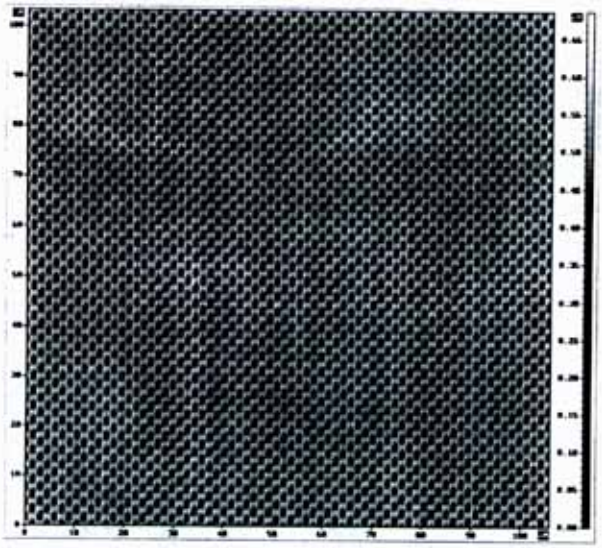

Figure 2. Image of the test 3 μm step grating.

to 30 μm) scanners are specially developed for inverted optical microscope Olympus IX-70, which is widespread in spectroscopy techniques. They are equipped with low voltage piezoelectric drives (0 to 100 V) of PI production integrated into a sophisticated flexure guiding system. The force exerted by the piezo drive pushes a multi-flexure parallelogram via an integrated motion amplifier. The wire EDM (Electric Discharge Machining) cut flexures are FEA (Finite Element Analysis) modeled for zero friction, ultra-high resolution and exceptional guiding precision. Integrated position feedback with the gauge sensor provides high resolution and stability in closed loop operation. To drive these scanners we use the modified typical electronic block for the "Solver" series SPMs of NT-MDT Co. production.

The modification of the electronic block consisted in adding new scheme with high-voltage amplifiers in the regime of the feedback with translation sensors. Therefore we can simultaneously control both XY-stage and usual SPM and there is a possibility to place the standard probe head "SMENA" at this stage. Using this construction, we can independently scan the sample in two ways either by moving the sample with the XY-scanner or with "SMENA" piezoscanner. The feedback on the relief is kept with "SMENA" Z-scanner and all modes (contact, tapping, Kelvin and MFM, etc.) are available.

The advantage of using XY-scanner consists in that fact that we always can move exactly to the needed point of the sample. This is especially important in

Technical characteristics of the	XY-scanner	Z-scanner
1. Max. scanning range, μm	$100 \pm 20\%$	$30 \pm 20\%$
2. Resolution, nm (closed/open loop)	2/1	2/1
3. Linearity, % (closed/open loop)	0.1/1	0.1
4. Reproducibility, nm	± 5	± 3
5. Max. Scanning Rate, Hz/line	20	20
6. Max. Weight, kg	2	0.3

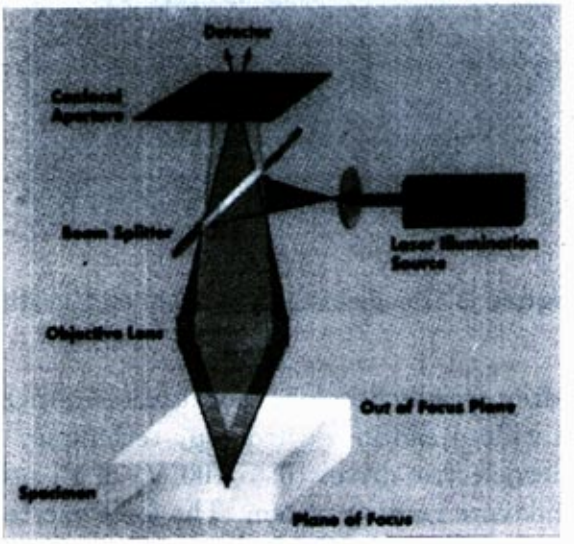

Figure 3. The principle of a confocal laser scanning microscope.

the scopes where we require the metrology fidelity, for instance, in microelectronics where the size of the element is approached to the nanometer scale. Figure 2 demonstrates the image of the test 3 μm grating obtained with XY-scanner.

The second spectrographic part is the confocal spectrometer with different detectors:

a. PMT R943-02

b. ICCD DH501

c. MCP-PMT R3809U-50 (optional)

d. CCD DV434-BU (optional)

In Fig. 4, the confocal principle is illustrated schematically for the fluorescence imaging mode [c.f. Wilson & Sheppard, 1984; Lichtman, 1994]. To image the specimen point by point, a collimated, polarized laser beam is deflected

stepwise in the x- and y-direction by a scanning unit (not shown) before it is reflected by a dichroic mirror (beam splitter) so as to pass through the objective lens of the microscope, and focused onto the specimen. The emitted, longer-wavelength fluorescent light collected by the objective lens passes through the dichroic mirror (transparent for the longer wavelength) and is focused into a small pinhole (i.e., the confocal aperture) to eliminate all the out-of-focus light, i.e., all light coming from regions of the specimen above or below the plane of focus. Therefore, the CLSM does not only provide excellent resolution within the plane of section, but also yields similarly good resolution between section planes. The in-focus information of each specimen point is recorded by a light-sensitive detector (i.e., a photo-multiplier) positioned behind the confocal aperture, and the analog output signal is digitized and fed into a computer. At the same time, the analog photo-multiplier signal can be used to generate a TV-like image on a video monitor. The obvious advantage of having a stack of serial optical sections through the specimen pixel by pixel in digital form is that a volume-rendered 3-D representation of the specimen can be generated.

The optical microscope Olympus IX-70 was combined with singular fully motorized (slit size, shutter, pinholes, output port, notch filter, and wavelength settings) monochromator which can be used in different modes (with or without grating). It is also possible to use lasers with different wavelengths (from ultraviolet 244 nm to infra 1050 nm) and both in CW and pulse regimes.

For Raman measurements we use 488 nm laser and the spatial resolution was about 150 nm (350–1050 nm spectral range, resolution of 0.19 cm^{-1}, and 8–60 μm pinhole).

The whole system is operated with the software developed by NT-MDT Co. It accomplishes initial approach, PMT scanning in analog signal mode, PMT scanning in photon counting mode, ICCD scanning (step by step and continuous), moving into certain position on the sample surface determined by the result of previous scanning, graphic software for image creation, 3D manipulation, and this is not the full list of its features. The signal from selected detector is measured in every XY point and then is shown as an image element. If the detector is CCD or ICCD then up to 20 parts of linear spectra can be preliminary selected and for every part we get 4 functions: integral intensity, peak intensity, position of the peak, and position of the center of gravity. Therefore for one exposure time we can get up to $4 \times 20 = 80$ images. Moreover, it is possible to scan the objective along the Z axis getting layer-wise images.

Some results obtained with Nanofinder.

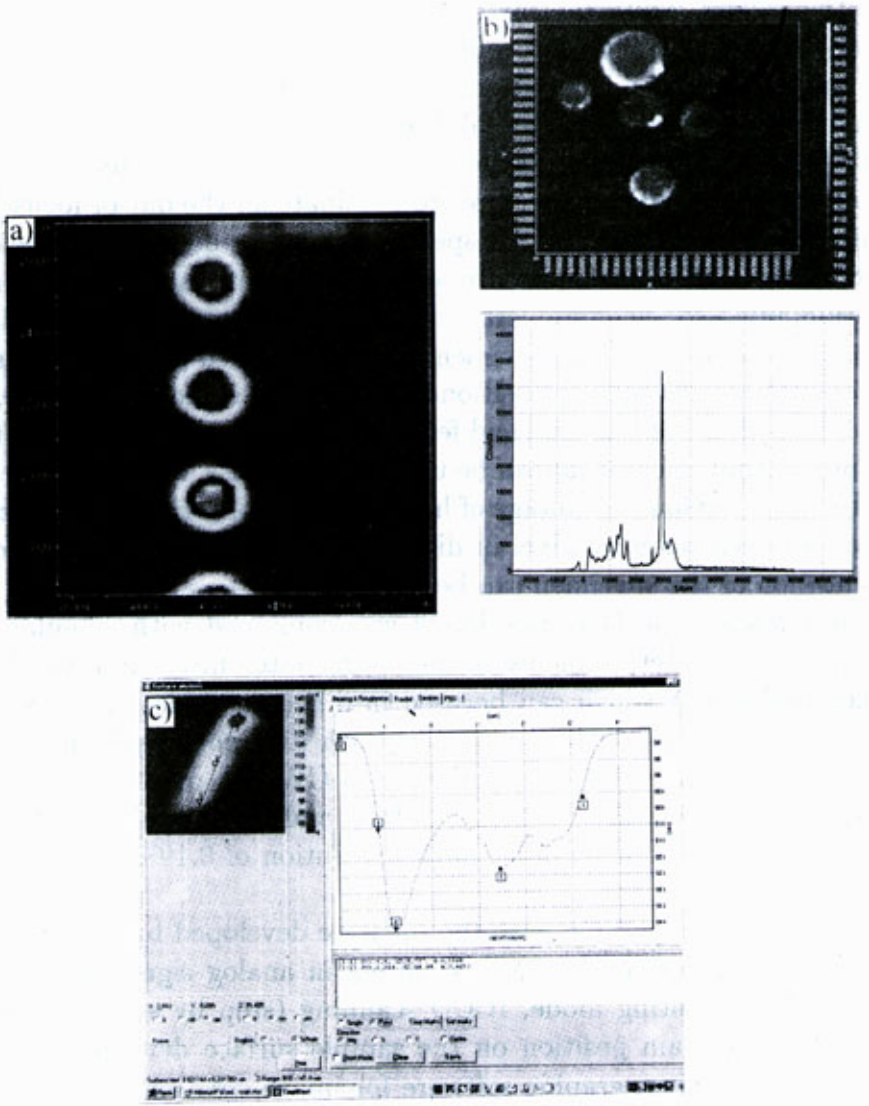

Figure 4. A) Raman image at ~3000 cm^{-1} and the spectrum of *vitamin*. B) Micromachining in fullerene film. Processing exposure time 5 seconds. Laser excitation at 488 nm, 1 mW. Fluorescent image in the 700–800 nm range. Exposure time 50 ms. Laser excitation at 488 nm, 0.01 mW. Scan size 10 × 10 μm. C) Dye (BCECF(2',7'-Bis(carboxyethl)-4 or 5-carboxyfluorescein)) distribution inside bacteria (Halobacterium salinarum). Laser excitation at 488 nm. Fluorescence signal at 510–520 nm.