

Applications of optical coatings on Raman spectrometer and biosensors

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Advances in optical coating technology over the past decade have made it possible to produce high-performance Raman spectroscopy filters with better reliability and at lower costs. The performance and characteristics of three typical Raman filters and an ultraviolet resonance Raman filter are introduced. Some applications of surface-enhanced Raman scattering (SERS) biosensors for the detection and identification of tissues, cells, proteins, nucleic acids, drugs, and chemical pathogens are reviewed.

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Raman spectroscopy was first described by C.V. Raman in 1928. In recent years, it has become a useful routine tool for the analysis and study of biological and pharmaceutical samples. Raman spectroscopy uses light scattered from an excitation laser to build up a chemical fingerprint characteristic of the sample. The Raman spectrum is composed by many “sharp” lines (Raman lines). The frequency shift between the excitation light and the Raman lines is determined by the energy of the molecular vibrations, which depends on the kinds of atoms, including their bond strengths and arrangements in a specific molecule. Further, it is extremely sensitive to the subtle changes in the chemistry of a sample. Since Raman spectroscopy is a technique that uses light irradiation, it can be adapted for using on a microscope. In the last few years, Raman microscopy has become apparent, coupling chemical information with microscopic scale. The new generation of confocal Raman microscopes have the capability to discriminate the area of the sample being analyzed very precisely, thus determine the chemistry of individual particles or cells. Because of these features, Raman spectroscopy has the potential to become a key biosensor for health monitoring based on molecular information. The high molecular specificity and the high content of chemical information of the technique likewise make it a very useful tool for the environmental control^[1].

The main advantage of Raman spectroscopy is its capability to provide rich information about the molecular structure of the sample. Recently, sophisticated-data-analysis techniques based on multivariate analysis made it possible to exploit the full information content of Raman spectra and draw conclusions about the chemical structure and composition of very complex systems such as biological materials. However, a great disadvantage in any applications of Raman spectroscopy comes from the extremely small scattering cross-section of the effect, resulting in very weak signals. Generally, there are two ways to enhance the sensitivity of Raman spectroscopy: developing a highly effective high-throughput optical system and inducing enhancement effect. With advances in laser technology such as narrow beam near-infrared (NIR) laser diodes, and charge-coupled device (CCD)

detector technology such as open electrodes, deep depletion, and back-illuminated chip formats, especially in Raman filters and other types of optical coatings such as image antireflection (AR) and laser mirror, the sensitivity of the latest Raman microscopes enables analysis and images to be respectively done and obtained far more quickly than previously possible. In the second way, by using selected ultraviolet (UV) excitation, resonance enhancement effect can increase Raman signal by many orders of magnitude. Further, with the interaction between sample molecules and metal nanostructures in surface-enhanced Raman scattering (SERS), signals can be enhanced up to 14 orders of magnitude.

In Raman spectroscopy application, an intense laser beam is used to create Raman scattered light from a sample under test (see Fig. 1)^[2]. The Raman “fingerprint” is measured by a dispersive or Fourier-transform spectrometer. Optical filters are critical components in Raman spectroscopy systems to prevent all undesired light from reaching the spectrometer and swamping the relatively weak Raman signal. Laser-transmitting filters inserted between the laser and the sample block all undesired light from the laser (such as broadband spontaneous emission or plasma lines) as well as any Raman scattering or fluorescence generated between the laser and the sample (as in a fiber-probe system). Laser-blocking filters inserted between the sample and the spectrometer block the Rayleigh scattered light at the laser wavelength.

A number of years ago, state of the art Raman filters were advanced by the introduction of volume holographic

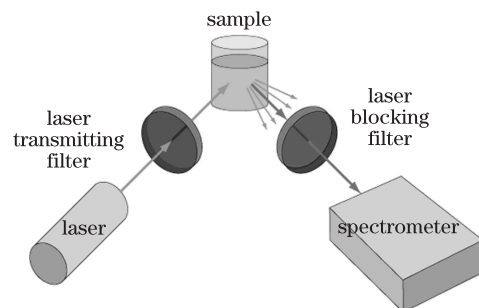


Fig. 1. Diagram of Raman spectrometer.

grating filters instead of multi-grating low-throughput systems to get rid of the Rayleigh scattering line of a laser. Holographic notch filters accomplish laser blocking by diffracting a spectral notch around the laser wavelength at an acute angle relative to the direction of the desired transmitted light. They also can serve as laser-line filters where the desired laser light is diffracted at an oblique angle, and a carefully aligned slit or pinhole is used to block unwanted light. The holographic gratings are exposed and developed in a thick gelatinous film that is typically sandwiched between two glass substrates. Unfortunately, these filters can be prohibitively expensive because they are manufactured one at a time^[3,4].

Recent advances in thin-film interference filter technology have made it possible to produce the highest-performance optical filters for Raman spectroscopy applications with higher reliability and at lower cost. These new filters are manufactured using ion-beam sputtering deposition, a process perfected for coating precise ferrite thin films on magnetic disks, extremely low-loss mirrors for ring-laser gyro applications, and high-performance optical filters for dense wavelength division multiplexed (DWDM) fiber-optic communications systems. This filter technology took a revolutionary step forward as a result of developments driven by the challenging requirements of DWDM.

There are four basic types of filters: a long wave pass (LWP) edge filter, a short wave pass (SWP) edge filter, a notch filter, and a laser-line filter. The laser-line filter is an obvious choice for the laser-transmitting filter, and the notch filter is an obvious choice for the laser-blocking filter. In systems using these two filter types, both Stokes

and anti-Stokes Raman scattering can be measured simultaneously. However, in many cases, the edge filter provides a superior alternative for both transmitting and blocking filters. Edge filter offers better transmission, higher laser-line blocking, and steeper edge performance to see Raman signals extremely close to the laser line. The examples in Fig. 2 show how the various filters are used.

Figure 3 illustrates the relative advantages of edge and notch filters^[5]. Figure 3(a) shows filter transmission on a linear scale and illustrates the capability of a LWP edge filter to get extremely close to the laser line. Figure 3(b) (where optical density (OD) is defined as $OD = -\lg(\text{transmission})$) shows the increased edge steepness of an edge filter relative to a notch filter. Increased edge steepness enables a narrower “transition width,” which is defined as the guaranteed maximum spectral separation between the laser line and the transmitting region of the filter spectrum for light normally incident on the filter. This is very important for low

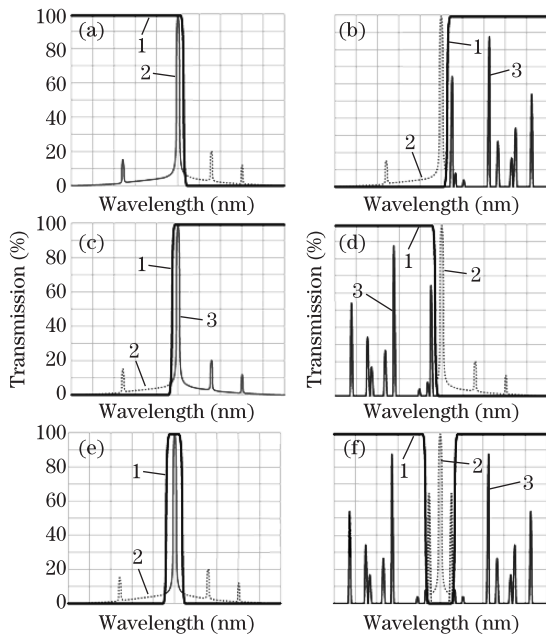


Fig. 2. Transmission spectra of (a) SWP and (b) LWP edge filters for detecting Stokes lines; transmission spectra of (c) LWP and (d) SWP edge filters for detecting anti-Stokes lines; transmission spectra of (e) laser-line filters for laser transmission and (f) notch filters for laser blocking under simultaneous Stokes and anti-Stokes measurements. The lines labeled with 1 represent the filter transmission spectra; the lines labeled with 2 represent the laser spectra, and the lines labeled with 3 represent the Raman signal^[2].

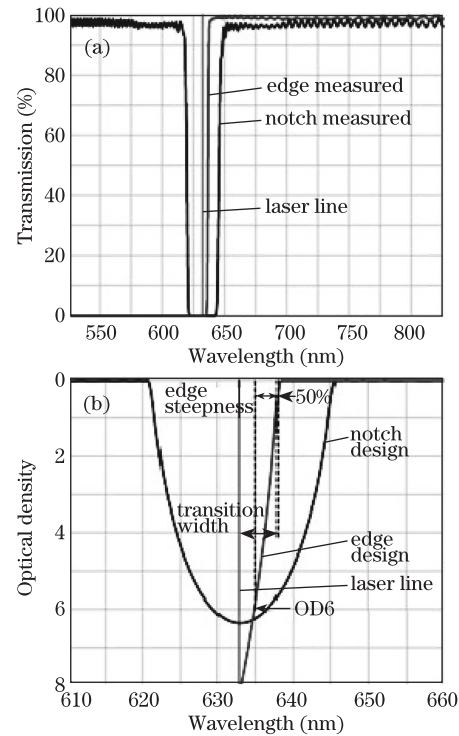


Fig. 3. Filter transmissions on (a) linear and (b) logarithmic scales^[5].

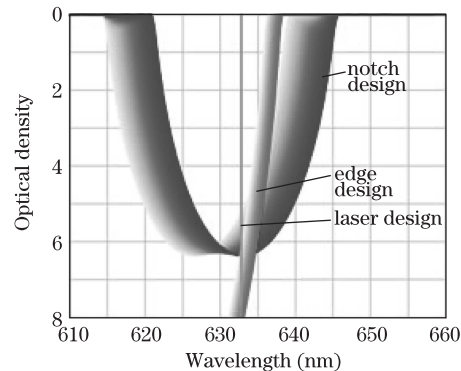


Fig. 4. Relative tuning ranges can be achieved for edge and notch filters^[5].

wave number applications^[6]. With transition widths below 1% of the laser wavelength, these filters do not need to be angle tuned.

Figure 4 shows the relative tuning ranges that can be achieved for edge and notch filters. Edge filters can be tuned up to 0.3% of the laser wavelength. The filters shift towards shorter wavelengths as the angle of incidence is increased from 0° to about 8°. Notch filters can be tuned up to 1.0% of the laser wavelength. These filters also shift towards shorter wavelengths as the angle of incidence is increased from 0° to about 14°.

Raman spectroscopy measurements generally face two limitations: 1) Raman scattering cross-sections are tiny, requiring intense lasers and sensitive detection systems just to achieve enough signal, and 2) the signal-to-noise ratio is further limited by fundamental, intrinsic noise sources such as sample auto-fluorescence. Raman measurements are most commonly performed with green, red, or NIR lasers largely because of the availability of established lasers and detectors at these wavelengths. However, by measuring Raman spectra in the UV wavelength range, both limitations can be substantially alleviated. Visible (Vis) and NIR lasers have photon energies below the first electronic transitions of most molecules. However, when the photon energy of the laser lies within the electronic spectrum of a molecule, as in the case of UV lasers and most molecules, the intensity of Raman-active vibrations can increase by many orders of magnitude—this effect is called “resonance-enhanced Raman scattering.” Further, although UV lasers tend to excite strong auto-fluorescence, this typically occurs only at wavelengths above 300 nm, independent of the UV laser wavelength. Since even a 4000-cm⁻¹ (very large) Stokes shift leads to Raman emission below 300 nm when excited by a common 266-nm laser, auto-fluorescence simply does not interfere with the Raman signal, making high signal-to-noise ratio (SNR) measurements possible^[7,8].

Recently, an increasing number of compact, affordable, and high-power UV lasers have become widely available, such as quadrupled, diode-pumped Nd:YAG lasers at 266 nm, and NeCu hollow-cathode metal-ion lasers at 248.6 nm, making ultrasensitive UV Raman spectroscopy now a widely accessible technique. Optical filters have continued to be a limitation, however. Some of the most challenging applications for optical filters in mid-UV are Raman and native-fluorescence “standoff” (remote) detection and classification of trace levels of chemical hazards. When excitation wavelengths are near or below 250 nm, Raman and native-fluorescence emission bands occur in different spectral regions for nearly all materials, enabling sensors that employ exclusive detection of Raman signals with no fluorescence interference or even simultaneous detection of both Raman and native-fluorescence emissions to identify unknown substances. Detection in mid-UV takes advantage of resonance signal enhancements from a wide array of organic materials, virtually eliminates fluorescence background emission from the target or surrounding materials, and enables solar-blind standoff detection during full sunlight conditions.

Success in achieving these goals depends heavily on the capability of optical filters to block scattering at laser excitation wavelength (OD > 6), to transform sharply from the blocked laser wavelength to high transmission in the

Raman detection bands, block unwanted light above the Raman and fluorescence bands to the long wavelength extent of the detectors used in the sensor, and exhibit no intrinsic fluorescence when exposed to mid-UV laser wavelength. For mid-UV edge filters, the transition from high blocking to high transmission must occur in typically less than 3–4 nm to achieve detection of Raman scattering less than about 500 cm⁻¹. A small amount of filter leakage at any undesired wavelength can seriously degrade the performance of the sensor, especially for spontaneous Raman scattering. Figure 5 shows an example of the measured transmission spectrum of a steep-edge filter made by modern ion-beam sputtering deposition and hard-oxide coating materials, overlaid on representative spectra (not to relative vertical scale) of a 266-nm laser line, several Raman lines, and near-UV and Vis auto-fluorescence.

SERS is another enhancing effect that combines the interesting optical properties of metal nanostructures with Raman spectroscopy. Because of resonances between the optical fields and the surface plasmon in metallic nanostructures, strongly enhanced local optical fields can exist at the very close vicinity of these structures. Raman scattering signals from molecules attached to nanometer-scaled silver and gold particles can be enhanced up to 14 orders of magnitude^[9].

SERS is not a resonance enhancement process; therefore, the excitation wavelength is a “free parameter” that can be selected depending on the specific application. The non-resonance excitation also avoids photodecomposition of UV lasers, since the wavelength of an excitation laser is not limited by the first electronic transitions of Raman molecules.

Molecular structural information, including monitoring structural changes, plays an increasingly important role in health monitoring as this kind of information can provide a deeper insight into the development and treatment of diseases as well as advanced early diagnosis based on a “molecular understanding.” Sensors that provide high molecular structural information content together with high sensitivity are of particular interest. There is also a strong interest in collecting spectroscopic information from small volumes, where biochemical modifications may start as a precursor of the development of diseases. SERS nano-sensors can address most of these requirements.

The potentials of the technique in the biophysical/biomedical field appear to be enormous (for example,

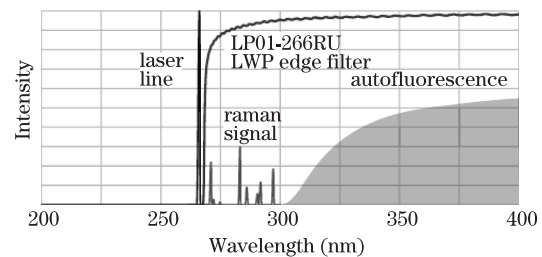


Fig. 5. New steep and highly transmitting LWP edge filter blocks the 266 nm line of a quadrupled Nd:YAG laser with OD greater than 6, while transmitting Raman signals from wavelengths as short as 268–300 nm and fluorescence at near-UV and Vis wavelengths^[5].

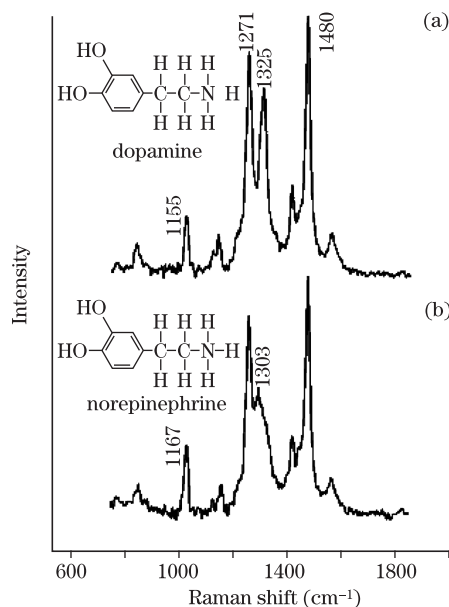


Fig. 6. SERS spectra of two neurotransmitters, dopamine and norepinephrine, in nanomolar physiologically relevant concentrations. Despite the very similar chemical structures, due to the high structural specificity of SERS sensors, both neurotransmitters show clearly different spectroscopic signatures^[1].

in the trace detection, identification, and quantification of biomedically relevant molecules such as neurotransmitters based on SERS signals). Such information can be of importance in case of emergency treatments (see Fig. 6).

Therefore, Raman has the potential to detect and image even the subtlest changes in chemistry or structure. For a long time, fluorescence microscopy has been a powerful tool for imaging cells and tissues by using fluorophores as tags to label bio-molecules for detecting biological states and mechanisms. However, what fluorescence microscopy does not reveal is precisely what chemistry may be involved or the discreet changes in composition or molecular structure. For instance, Raman has been used to produce chemical images of cells and biological tissues where diseased states may be detected. An example of this has been in the analysis of cancerous tissues. The pathological state of tissue showing advanced malignancy is easy to determine through conventional microscopy of a histological sample, but for cases where the cancer is far less advanced or in its earliest stages, visual changes in the cell become more ambiguous. Actually, even at this early stage, the chemistry has begun to change, and Raman has recently been shown to be capable of detecting this subtle change. It can also be used to image the localization of further diseased states or active drug therapies within cells, elucidating where a drug should be targeted or where it may migrate.

As a spectroscopic technology, SERS combines the advantages of fluorescence spectroscopy and Raman spectroscopy as illustrated in Table 1^[1]. Additionally, since SERS spectroscopy takes place in local optical fields, lateral confinement is determined by the confinement of the local fields, allowing the collection of spectroscopic data from volumes below 5-nm dimensions.

Table 1. SERS Combines the Advantages of Fluorescence and Raman Spectroscopy

	Information Content	Sensitivity
Raman Scattering	High	Low
Fluorescence	Low	High
SERS	High	High

Currently, the detection of protein and chemical pathogens (targets) is most commonly done using fluorescence detection of target-receptor binding. Although very sensitive, fluorescence detection suffers from the need to label the molecular target, which can also lead to alterations in target-receptor interactions caused by conformational changes or steric hindrance induced by the label. For these reasons, there is considerable effort to investigate label-free sensors using a variety of methods including optical, mechanical, and electrochemical techniques. Surface plasmon resonance sensing is one promising optical label-free technique. Another compelling technique measures SERS spectra from the target biomolecules. Therefore, this label-free optical sensing technique has the capability to quantify molecules attached to the sensing surface, which should improve the well-known problem of non-specific binding in all solid surface sensing systems. Additionally, it is desirable to have small, rapid assays that use small sample volumes and are capable of detecting several compounds of species in parallel. This is significant because in many cases, sample collection is limited, and sample processing also requires time. All of these factors point towards the capability to detect multiple agents in parallel using small sample volumes. In this kind of application, Raman micro-spectroscopy can be a reader of biosensors or so-called biochips.

In Raman micro-spectroscopy, a spectrometer is coupled to an optical microscope to enable both excitation and collection of Raman spectra. Additionally, the high quality of optical microscopes make it possible to obtain measurements with diffraction-limited spatial resolution. Spatial resolution along the optical axis can be improved by using a confocal setup, which employs a small pinhole (less than 100 μm) in front of the detector to reject the out-of-focus photons. While certain microscopes use such pinholes, other designs use the actual detector pixels or the aperture of the optical fiber collection as a confocal pinhole. Confocal setups can report sampling volumes as small as 1.4 fL from inside single cells.

The first requirement for a biosensor is the capability to discriminate between healthy and dead cells^[10]. To test this capability, Raman spectra of healthy and dead cells were compared to identify the main spectral differences. Figure 7 shows the micrographs of individual healthy and dead cells after the Raman spectra were measured. The comparison between the measured Raman spectra of healthy live cells and dying cells revealed significant differences that were used as markers of cell viability.

SERS is a technique that has also been used for analyzing the intracellular interactions of the anticancer drug mitoxantrone^[11]. However, in addition to studying these fundamental properties, work has also focused on disease diagnosis of tissues, where the capability of Raman spectrum to pick up even very subtle changes in

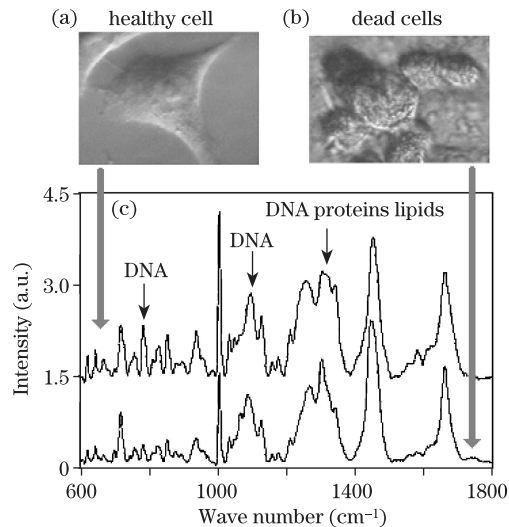


Fig. 7. Comparison between the Raman spectra of healthy and dead cells. Significant differences are observed related to DNA, proteins, and lipids^[10].

tissue biochemistry allows healthy and diseased tissues to be accurately distinguished. It is clear that the Vis structural features of the tissue coincide with the chemical information provided by the Raman spectrum. Cluster analysis of the Raman spectrum data allows spectra of similar profile to be grouped and displayed as a single unit, allowing specific tissue types to be located. This technique makes use of the large information content present even in just one spectrum, which will give an indication of the presence of the various chemicals present within the tissue, such as DNA, RNA, protein, lipid, and carbohydrate. Comparison between data obtained from healthy and diseased tissues enables scientists to not only learn more on the biochemical changes caused by the cancer but also categorize tissue according to disease state.

In conclusion, the potential market for ultrasensitive sensors in life sciences, health monitoring, and environmental analysis and control is enormous, particularly

because there is a strong need for structurally sensitive technologies, a field that cannot be covered by existing spectroscopic or optical solutions. However, there is no competing technology to date that can provide the high content of structural molecular information of a SERS sensor. Optical coatings still play an important role in the market of SERS biosensors.

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References

1. K. Kneipp and H. Kneipp, *CA- NEUS 2004 Conference on Micro-Nano-Technologies for Aerospace Applications from Concepts to Systems*, 56 (2004).
2. Semrock, Inc. "Filter types for raman spectroscopy applications", http://www.semrock.com/TechnicalInformation/TN_Raman_filtertypes/ (January 15, 2008).
3. T. Erdogan and V. Mizrahi, *Spectroscopy* **19**, 113 (2000).
4. T. Erdogan and V. Mizrahi, *Photon. Spectra* **37**, 94 (2003).
5. Semrock, Inc. "Edge Filters vs. Notch Filters for Raman Instrumentation", http://www.semrock.com/TechnicalInformation/TN_Raman_edgevsnotch/ (January 15, 2008).
6. M. Ivanda, K. Furic, S. Music, M. Ristic, M. Gotic, D. Ristic, A. M. Tonejc, I. Djerdj, M. Mattarelli, M. Montagna, F. Rossi, M. Ferrari, A. Chiasera, Y. Jestin, G. C. Righini, W. Kiefer, and R. R. Goncalves, *J. Raman Spectrosc.* **38**, 647 (2007).
7. T. Erdogan and A. Pradhan, *Photon. Spectra* **42**, 76 (2008).
8. T. R. Jensen, R. L. Johnson, Jr., J. Ballou, W. Prohaska, and S. E. Morin, *43rd Annual Technical Conference Proceedings of the SVC* 239 (2000).
9. X. Zhang, C. R. Yonzon, M. A. Young, D. A. Stuart, and R. P. Van Duyne, *IEE Proc.-Nanobiotechnol.* **152**, 195 (2005).
10. I. Notingher, *Sensors* **7**, 1343 (2007).
11. S. F. Gerald, *BIO forum Europe* **9**, 56 (2005).