Chevron-Type Dielectric Filter Set for Efficient Narrow-Band Laser Line Rejection in Raman Microspectrometers

GERWIN J. PUPPELS, CEES G. de GRAUW, MAURICE B. J. te PLATE, and JAN GREVE*

Department of Applied Physics, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands

A chevron-type dielectric bandpass filter set is described which combines laser line rejection by a factor $>10^8$ with a high throughput of Raman scattered light (70%). The rejection bandwidth is 60 cm^{-1} full width at half-maximum. Stokes and anti-Stokes Raman spectra can be recorded simultaneously from approximately 20 cm^{-1} from the laser line. The filter set, moreover, takes care of efficient coupling of microscope and spectrometer, replacing an otherwise necessary beamsplitter.

Index Headings: Stokes; Anti-Stokes; Chevron-type dielectric filter.

INTRODUCTION

Multichannel detection with intensified photodiode arrays or liquid nitrogen (or thermoelectrically) cooled charge-coupled device (CCD) cameras has enormously increased the sensitivity of Raman spectroscopy. From the first applications,[1] efficient laser light suppression in a multichannel Raman setup has been a point of concern. In order to simultaneously image a significant part of the Raman spectrum on the detector and fully exploit the multichannel detection advantage, a relatively low wavelength dispersion is needed in combination with a strong suppression of the laser line. Triple-grating-stage spectrometers, in which the first two grating stages serve to suppress laser light intensity, provide good laser line suppression, usually allowing measurements starting at about 20 cm^{-1} from the laser line without any problem. Moreover, they can be tuned to work with different laser excitation wavelengths. However, in general, they also have a rather low Raman signal throughput, on the order of 10% ($\sim$50% signal throughput per grating stage), and this becomes worse when the setup is tuned for use with a laser wavelength away from the blaze wavelength of the gratings.

Alternative laser line rejection methods have been realized, aimed at improving the Raman signal throughput. Ideally a laser light suppression filter would completely block elastically scattered and reflected laser light. It would fully transmit Raman scattered light starting from a wavenumber shift where, taking the spectral linewidth of the laser source into account, Raman signal is, in principle, detectable. Colored-glass cutoff filters,[2] which allow Stokes–Raman signal detection from about 800 cm^{-1}, and colloidal Bragg reflection filters,[3] allowing both Stokes and anti-Stokes Raman measurements, have been used. Among the more recent developments are Raman holographic notch filters[4–6] [optical density (O.D.), $\sim$5–6; rejection band full width at half-maximum (FWHM), $\sim$300 cm^{-1}; signal transmission, $\sim$80%], volume holographic narrow-band optical filters,[7] and atomic vapor

filters.[8,9] The latter can provide very strong laser attenuation—up to 10 O.D. units in a very narrow rejection band of only a few wavenumbers. Some practical problems with atomic vapor filters are the occurrence of absorption bands in other parts of the spectrum and vapor resonance fluorescence.

Recently we developed a chevron-type dielectric notch filter set with very strong laser attenuation (O.D. = 8).[10] It has been used in a confocal Raman microspectrometer, which allowed the recording of Raman spectra of single living cells and chromosomes.[11] Here we report on a version of the chevron-type Raman notch filter set especially developed to allow measurements close to the exciting laser line. It has an FWHM of the laser light rejection band of 60 cm^{-1} and will be employed to record low cm^{-1} Raman-active modes of DNA and proteins in cells and chromosomes.

MATERIALS AND METHODS

Two identical bandpass filters, with a 75% transmission band of 0.5-nm bandwidth (FWHM) at 661 nm for perpendicularly incident light (i.e., an FWHM of 10 cm^{-1}), were purchased from Omega Optical, Inc. From about 3 nm to both the Stokes and anti-Stokes sides of the transmission maximum, the filters have a $>$97.5% reflection.

DESIGN

Principle. The principle of the Raman notch filter presented here (Fig. 1) is similar to that of the chevron-type filter set described earlier. A collimated beam of light coming from the sample is reflected back and forth between two parallel ultra-narrow dielectric bandpass filters (FWHM is 0.5 nm and central wavelength is 661 nm, with normal incidence). The normal to the filter surface makes a small angle ($\sim$1°) with the beam of light. The laser wavelength is tuned in such a way that laser light is optimally transmitted ($\geq$70% transmission) by the filters under this angle of incidence. This means that with each reflection $\geq$70% of the laser light is effectively removed from the beam of light, whereas Raman shifted light is reflected. With a total of 16 reflections the laser light intensity is, in theory, attenuated by a factor $(0.3)^{16} \approx 5*10^{-9}$. From about 3 nm to either side of the central transmission wavelength, the filters have a reflection $>$97.5% so that more than $(0.975)^{16} \approx 70\%$ of the Raman signal enters the spectrometer, which consists of a single-grating stage and a liquid nitrogen-cooled slow-scan CCD camera (Wright Instruments, Ltd.) fitted with a thinned back-illuminated Tektronix 512 TKB charge-coupled device. In the configuration shown in Fig. 1, the notch filter is not only used to suppress the intensity of Rayleigh
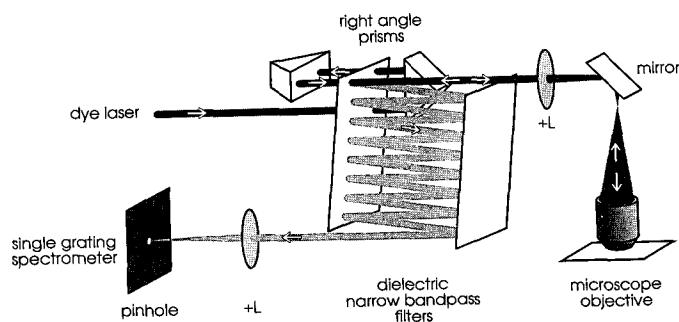
FIG. 1. Confocal Raman microspectrometer for simultaneous low-wavenumber Stokes and anti-Stokes Raman measurements. The notch filter set suppresses laser line intensity and optically couples microscope and spectrometer.

FIG. 3. Signal transmission curve of the notch filter set. [The suppression band does not reach zero in this curve. This result is due to the relatively low spectral resolution of the setup ($\sim$7–8 cm^{-1}) in its present form, which leads to the presence of stray (white) light at the position of the notch filter rejection band on the CCD camera.]

scattered and reflected laser light, but it also couples the microscope and the spectrometer, replacing an otherwise necessary beamsplitter. The laser beam enters the microscope after passing through the filter at the right in Fig. 1. In practice, two right-angle prisms are used in order to let the laser light pass through this filter three times before entering the microscope. This is done in order to prevent dye laser fluorescence from reaching the sample, which would make measurements in the low cm^{-1} region impossible (see Fig. 2).

Filter Angle. When dielectric filters are used under conditions of non-normal light incidence, filter characteristics undergo a shift to lower wavelength. This characteristic can actually be used for wavelength tuning of the filter.[10,12] However, the filter characteristics for p- and s-polarized light tend to diverge.[10] This behavior is especially problematic when one is using filters with an ultra-narrow bandpass such as in this work, because the

spectral overlap between the transmission bands for p- and s-polarized light rapidly diminishes for non-normal light incidence. Therefore, a configuration was chosen in which the normal to the filter surface makes an angle of only 1% with the incident beam of light, which does not lead to any noticeable divergence of the filter characteristics for p- and s-polarized light.

Pinhole. The setup is a confocal Raman microspectrometer. The pinhole which forms the entrance to the spectrometer ensures that only light originating from the laser focus in the sample will be detected.[13] Light scattered in the notch filter set, which could reduce its performance with respect to laser line suppression, is blocked.

PERFORMANCE

Determination of Raman Signal Throughput. Absolute Raman signal throughput through the filter set was determined in two steps. First the spectrum of the light from a tungsten band lamp (Philips 6002 E) fixed under the objective of the microscope and transmitted by the filter set (Fig. 1) was recorded. This spectrum was divided by the spectrum recorded with the filter set removed. The current through the band lamp was kept constant during these measurements (16 A). Care was taken to ensure that the temperature (2130 $\pm$ 30 K) of the tungsten band which determines intensity and spectral distribution of the emitted light remained stable during the measurements by means of a pyrometer (Hartmann & Braun). An absolute scale for the transmission curve of the notch filter set was determined by measuring the absolute throughput at one wavelength. To this end, a laser diode, emitting at 673 nm (Philips CQL80D-0395), was placed in the focus of the microscope objective, and the intensity of the laser beam entering and leaving the notch filter set was measured with a power meter (Newport, Model 835). Figure 3 shows the absolute signal transmission of the filter set as a function of relative wavenumber ($\lambda_{laser} = 661$ nm). The FWHM of the laser line suppression band is 60 cm^{-1}, $\sim$60–70% transmission is maintained until 2000 cm^{-1} on the Stokes and anti-Stokes sides. Above 2500 cm^{-1}, transmission rapidly decreases to about 15% at 3000 cm^{-1} and <5% above 3200 cm^{-1}.
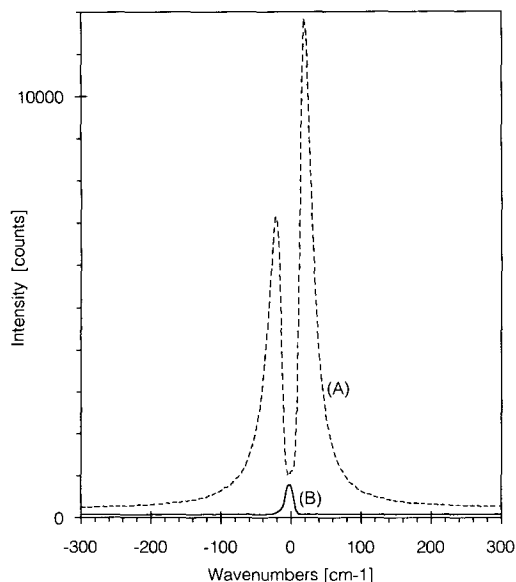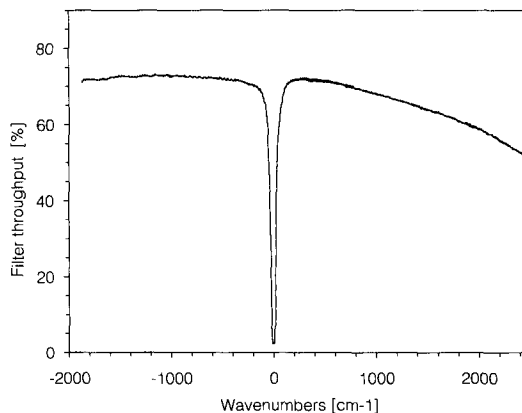

FIG. 2. Suppression of dye laser fluorescence, by guiding the laser light three times through one of the filters of the notch filter set before directing it to the microscope. Spectrum A was recorded by reflecting laser light from a mirror under the microscope without using the right-angle prisms (i.e., light from the dye laser passes only once through the dielectric filter, before being coupled into the microscope). Spectrum B was recorded with the right-angle prisms installed. Fluorescence from the dye laser is fully suppressed.
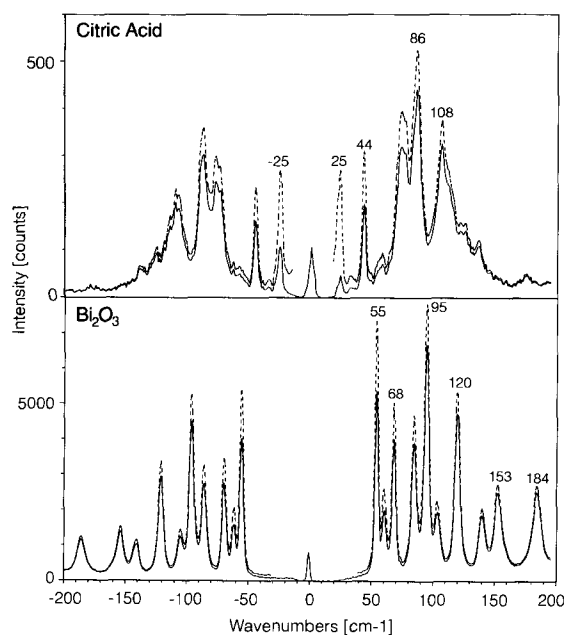
FIG. 4. (Top) Simultaneously recorded Stokes and anti-Stokes Raman spectra of citric acid. (Bottom) Simultaneously recorded Stokes and anti-Stokes Raman spectra of bismuth oxide. Solid lines: spectra not corrected for wavenumber dependence of signal detection efficiency; dashed lines: same spectra after correction; see text. Experimental conditions for both spectra: signal integration time, 30 s; laser power on sample, 10 mW, ×63 Spindler & Hoyer microscope objective (0.85 N.A.).

Determination of Laser Light Suppression. With the use of the configuration of Fig. 1, without the right-angle prisms, laser light was focused on a mirror under the microscope objective. Laser intensities were measured by means of a Newport Model 835 power meter. The intensity of the reflected light reaching the CCD camera of the setup was measured for 661-nm laser light, which is optimally suppressed by the filter set, and for 650-nm laser light, which is optimally transmitted. For the experiment with 650-nm laser light, calibrated neutral-density filters were used to attenuate the intensity of the incoming laser light in order to avoid saturation of the CCD camera. The results were corrected for this factor and also for the difference in laser intensity on the mirror at the two wavelengths, due to the fact that laser light was coupled into the microscope through one of the filters of the notch filter set (high transmission at 661 nm but low transmission at 650 nm). The laser line suppression at 661 nm that was determined in this way was equal to the theoretical value given above: $\sim 5*10^{-9}$.

CORRECTION OF MEASURED LINE INTENSITIES FOR FILTER CHARACTERISTICS

Recorded line intensities can be corrected for the wavenumber dependence of the detection efficiency of the setup, which in the low-wavenumber region is mainly determined by the transmission characteristics of the notch filter set. A correction spectrum is calculated by dividing the recorded emission spectrum of a tungsten band lamp of known temperature by the theoretical band lamp emission spectrum (given by Planck's formula for blackbody radiation). Measured Raman spectra are divided by this
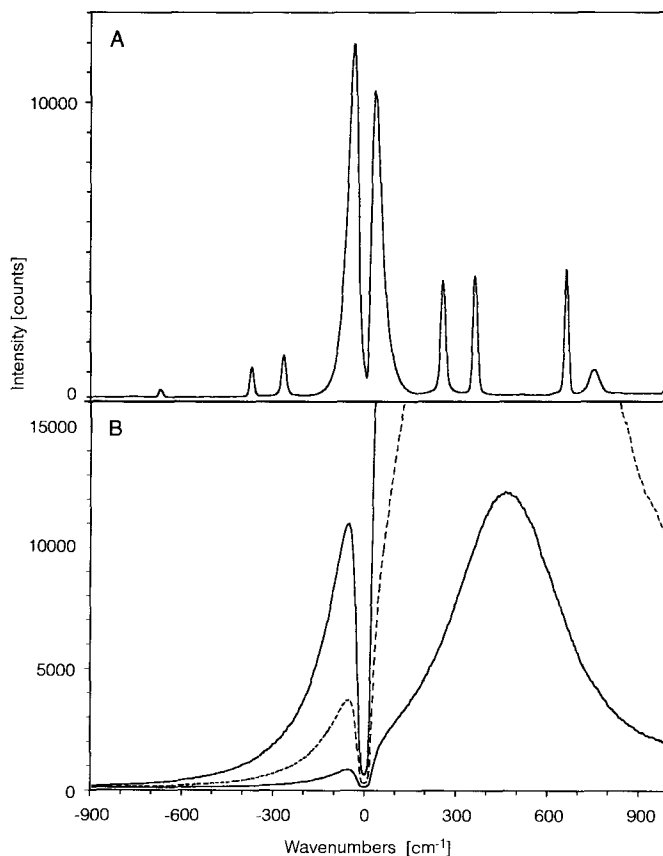

FIG. 5. (A) Simultaneously recorded Stokes and anti-Stokes Raman spectrum of chloroform. (B) Simultaneously recorded Stokes and anti-Stokes fluorescence spectrum of AlTsPc. Note the complete absence of the exciting 661-nm laser line in the recorded spectra. Experimental conditions: samples were kept in square glass capillaries [inner dimension, 500 μm; wall thickness, 100 μm (Vitro Dynamics Inc.)]. Spectrum A: signal integration time, 10 s; laser power on sample, 10 mW; ×40 Spindler & Hoyer microscope objective (0.65 N.A.). Spectrum B: signal integration times, 20 ms (solid line), 100 ms (dashed line), and 300 ms (dotted line); laser power, 10 μW. The spectra have not been corrected for wavenumber dependence of signal detection efficiency.

correction spectrum in order to eliminate effects of the wavenumber-dependent signal throughput of the setup. The effect of this procedure is illustrated in Fig. 4.

EXAMPLES

Examples that illustrate the performance of the setup described here are shown in Figs. 4 and 5. Figure 5A shows a chloroform Raman spectrum. The suppression of the laser line is such that it is not visible in the spectrum. The notch filter characteristics also suppress the intensity of the chloroform low-wavenumber librational modes near the laser line. Figure 5B shows part of the fluorescence spectrum of tetra-sulfonated aluminum phthalocyanine (AlTsPc) excited at 661 nm. Some fluorescence intensity is present on the anti-Stokes side of the laser line, due to molecules that were initially in an excited vibrational state. In Fig. 4 spectra of citric acid and bismuth oxide (both dry samples) are shown. In both cases the spectrum before (solid line) and after (dashed line) correction for the wavenumber dependence of the detection efficiency of the setup are shown. Simultaneous Stokes

and anti-Stokes measurements are possible down to about 20 cm^{-1} from the laser line.

CONCLUSION

The filter set described here is not as easy to use as, for example, holographic notch filters, but performance is superior with respect to laser line suppression ($\geq 10^{-8}$ vs. 10^{-4}–10^{-6}) and in the low cm^{-1} region (FWHM ~ 60 cm^{-1} vs. ~ 300–350 cm^{-1}).[6,14] Both types of filters have the advantage over other laser line filters, such as, for example, the atomic vapor filters,[9] in that they can be used to optically couple microscope and spectrometer in an efficient way (i.e., without the use of a separate beam-splitter).

The present filter set was optimized with respect to low-wavenumber performance and turned out to have low transmission in the CH and OH stretching region. There are, however, no fundamental restrictions which stand in the way of further improvement of transmission above 3000 cm^{-1} if so desired. Also, further improvement of the filters with respect to reflectivity for Raman signal, to values $>99\%$, should be possible and will lead to a signal throughput of approximately 80 to 90%.[10]

1. R. Mathies and N.-T. Yu, J. Raman Spectrosc. 7, 349 (1978).
2. A. Deffontaine, M. Bridoux, M. Delhaye, E. Da Silva, and W. Hug, Revue Phys. Appl. 19, 415 (1984).
3. P. L. Flaugh, S. E. O'Donnell, and S. A. Asher, Appl. Spectrosc. 38, 847 (1984).
4. M. M. Carrabba, K. M. Spencer, C. Rich, and D. Rauh, Appl. Spectrosc. 44, 1558 (1990).
5. B. Yang, M. D. Morris, and H. Owen, Appl. Spectrosc. 45, 1533 (1991).
6. C. L. Schoen, S. K. Sharma, C. E. Helsley, and H. Owen, Appl. Spectrosc. 47, 305 (1993).
7. G. A. Rakuljic and V. Leyva, Opt. Lett. 18, 459 (1993).
8. M. J. Pelletier, Appl. Spectrosc. 46, 395 (1992).
9. M. J. Pelletier, Appl. Spectrosc. 47, 69 (1993).
10. G. J. Puppels, A. Huizinga, H. W. Krabbe, H. A. de Boer, G. Gijsbers, and F. F. M. de Mul, Rev. Sci. Instrum. 61, 3709 (1990).
11. G. J. Puppels, F. F. M. de Mul, C. Otto, J. Greve, M. Robert-Nicoud, D. J. Arndt-Jovin, and T. M. Jovin, Nature 347, 301 (1990).
12. D. N. Batchelder, C. Cheng, W. Mueller, and B. J. E. Smith, Makromol. Chem., Macromol. Symp. 46, 171 (1991).
13. G. J. Puppels, W. Colier, J. H. F. Olminkhof, C. Otto, F. F. M. de Mul, and J. Greve, J. Raman Spectrosc. 22, 217 (1991).
14. Holographic Notch and Supernotch Filters (Kaiser Optical Systems, Inc., Ann Arbor, Michigan).