Symmetrically Tapered <30-µm-thick Quasi-Planar Germanium Waveguides as Chemical Sensors for Microanalysis

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Symmetrically tapered planar IR waveguides have been fabricated by starting with a ZnS coated concave piece of single-crystal Ge, embedding it in an epoxide resin as a supporting substrate, and then grinding and polishing a planar surface until the thickness at the taper minimum is <30 µm. Such tapering is expected to enhance a waveguide's sensitivity as an evanescent wave sensor by maximizing the amount of evanescent wave energy present at the thinnest part of the waveguide. As predicted by theory, the surface sensitivity, i.e., the absorbance signal per molecule in contact with the sensing region, increases with decreasing thickness of the tapered region even while the total energy throughput decreases. The signal-to-noise ratio obtained depends very strongly on the quality of the polished surfaces of the waveguides. The surface sensitivity is superior to that obtained with a commercial Ge attenuated total reflection (ATR) accessory for several types of sample, including thin films (<10 ng) and small volumes (<1 µL) of volatile solvents. By using the waveguides, light-induced structural changes in the protein bacteriorhodopsin were observable using samples as small as ~50 pmol (~1 µg). In addition, the waveguide sensors can reveal the surface compositions on a single human hair, pointing to their promise as a tool for forensic fiber analysis.

Index Headings: Tapered Ge waveguides; Evanescent wave sensor; Throughput; Sensitivity; Nujol; D96N mutant; Bacteriorhodopsin (bR); Hair analysis.

INTRODUCTION

Attempts to develop compact chemical sensors based on optical fi bers and other types of miniature waveguides have been driven by increasing demands to handle very small-sized samples in microanalysis and trace analysis. The greatest progress on development of waveguide sensors based on vibrational spectroscopy has been in the near-infrared (NIR), due to greater manufacturing experience with silica and other optical glasses that transmit in the visible and NIR. Due to signifi cantly stronger and more easily interpreted fundamental absorption bands in the mid-infrared (MIR) region, as compared to the overtones or combination bands appearing in the NIR region, it is desirable to develop alternative waveguides for the MIR spectral range. MIR optical waveguides have been fabricated from IR-transparent materials with high refractive index such as germanium (Ge), zinc selenide (ZnSe), and chalcogenide.¹⁻¹¹ MIR optical sensors have been demonstrated to be a potentially powerful chemical

sensing tool, as seen in many publications over the past few years.¹²⁻¹⁸

Whether a cylindrical optical fiber or a planar waveguide in various shapes is utilized, the technique can generally be referred to as either attenuated total reflection (ATR) or evanescent wave absorption. This phenomenon is well known in MIR spectroscopy and has successfully become a widespread technique for both qualitative and quantitative measurements. In this case, the measured absorption spectrum is the result of attenuation of the evanescent wave accompanying an incident IR beam traveling through the high-index waveguide. At the interface of two media with different refractive indices, the evanescent field penetrates a fraction of a wavelength beyond the waveguide into the lower-index sample layer. It decays exponentially within a shallow region, defined by the penetration depth (d_p) . For MIR light, the penetration depth is generally in the range of $0.5-5 \ \mu m$ depending on both experimental conditions (e.g., angle of incidence) and material characteristics (i.e., refractive indices of waveguide and sample).¹⁹ In general, the limits of detection for various MIR sensing systems have been too high for use as a trace method. Hence, there is substantial interest in improvements in the design and optical configuration of waveguide sensors in order to enhance their sensitivity.

Use of some optical fibers made of IR-transmitting glasses have been published.²⁻¹¹ More recently, methods of fabricating supported thin planar germanium (Ge) waveguides have already been published by our laboratory.²⁻⁸ These ~50- μ m-thick supported planar Ge waveguides have also previously been presented as chemical tools for applications with biomolecules,^{3,4} but the use of IR microscopes with the waveguide created a substantial restriction on sample handling and also limited the waveguide length to the range of <12 mm, the maximum adjustable distance between the focal points of objective and condenser in commercial FT-IR microscopes. In order to eliminate the IR microscope, several new designs of thin Ge waveguides have been presented that rely on particular fabrication processes.^{7,8}

One diffi culty in fabricating Ge waveguides as thin as $<30 \ \mu m$ is the poor mechanical stability of Ge, i.e., it can be easily broken during a grinding or polishing process. However, its property of chemical inertness, particularly with biochemical molecules in picomolar quantities, is a continued impetus for choosing this material. For development studies, an additional advantage is the

Received 13 May 2002; accepted 18 August 2002.

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FIG. 1. Schematic configuration of the <30-µm-thick symmetrically tapered Ge waveguide supported by epoxide substrate and used as miniature internal reflection element (IRE). The top planar surface of the waveguide acts as a sensing area, while its bottom is a cylindrical sector with a diameter of 600 mm, coated with a 2-µm-thick ZnS cladding layer. Incident light, indicated at left, was typically generated from the IR source of an FT-IR spectrometer, and the output was coupled onto a liquid N₂ cooled HgCdTe immersion detector placed as close as possible to the exit end of the waveguide. The detector window, outside the lens, is not shown.

presence of characteristic high- and low-frequency Ge cut-offs at 5200 and 670 cm⁻¹, respectively. The presence of these cut-off frequencies provides useful evidence for propagation of broadband IR light through the wave-guide.

In the work described here, we reduce the sensor-region thickness of symmetrically tapered Ge waveguides (see Fig. 1) to 7–30 μ m in order to achieve further sensitivity enhancements. Tapering allows the use of common focusing optical elements without an IR microscope. Substantially higher total energy throughput can then be achieved with very simple optical alignment procedures, as illustrated in Fig. 1. The tapered waveguides have a planar upper surface and a gently curved lower surface that is parallel to the upper surface only near the line segment corresponding to the thinnest region of the waveguide. Lacking a planar lower surface, these waveguides do not meet the standard definition of planar waveguides; but because of their near-parallelism at the sensing region, we refer to them as quasi-planar.

Tapering to such a thin dimension is expected to result in enhanced sensitivity through an increase in the detected absorption intensity per unit area of sample-waveguide contact. The challenge addressed in the current work was in maintaining adequate throughput of light to be able to take advantage of the high surface sensitivity. We present a number of potential new applications for the waveguide sensors, including light-dark difference spectroscopy of picomolar quantities of the purple membrane protein, bacteriorhodopsin (bR), and the nondestructive surface layers of short pieces of human hairs.

EXPERIMENTAL

Waveguide Fabrication. The raw material utilized for fabrication of waveguides was a 70-mm-diameter, 3-mmthick single-crystalline Ge disk (Lattice Materials Corp., Bozeman, MT). The disk was first symmetrically tapered and polished on a grinding machine (K & S Optics, Binghamton, NY) with a cylindrical aluminum grinding tool with a 600-mm curvature diameter, producing a curved surface on one side of the disk as depicted in Fig. 1. The polished curved surface was then coated with a 2- μ m-thick ZnS cladding layer by using a chemical vapor deposition (CVD) process. This cladding layer prevents IR light from being absorbed by the supporting substrate. The ZnS coated round Ge disk was then diced into 2-mm-wide strips using a vertical band saw (Materials Facility, Cornell Center for Materials Research, Cornell University, Ithaca, NY). Each of these strips was ground on its two parallel planar sides, to a fi nal width of 0.5-1 mm.

In the next step, each ZnS coated tapered Ge strip was individually embedded into epoxide resin (Epothin®, Buehler Ltd., Lake Bluff, IL) by pouring a well-blended mixture of epoxide resin and hardener in the manufacturer's recommended proportion onto the Ge strip held, flat side down, in a disk-shaped mold. After the epoxide resin was completely polymerized and fully hardened, the bottom (flat) side of the Ge and epoxide was ground and polished to the desired thickness, i.e., <30 µm. For experiments to determine the effect of surface-fi nish quality on waveguide transmittance, hand grinding and polishing utilized various polishing compounds and polishing disks manufactured by Buehler Ltd. (Lake Bluff, IL). For all other experiments, in order to obtain a high-quality fi nish, waveguides were sent to a commercial optics house (K & S Optics, Binghamton, NY) equipped with automated grinding/polishing machines.

The quality of spectra taken with these tapered Ge waveguides depends strongly on the quality of the final polished sensing surfaces because surface scratches cause light scattering and thereby result in less total energy throughput and a correspondingly poor signal-to-noise (S/N) ratio. The thickness of the waveguide was determined periodically during grinding and polishing by rinsing and drying the waveguide, then measuring the interference pattern (``channeling") that appears on the single beam spectrum taken transverse to the thinnest part of the waveguide with an IR microscope (IR-Plan Infrared Microscope Accessory, Spectra-Tech, Stamford, CT) connected to an FT-IR interferometer (Illuminator, Midac Corp., Irvine, CA) in reflectance mode with a 1 cm⁻¹ spectral resolution and 256 scans.²⁰

Waveguide Optical Alignment. Figure 1 shows a schematic of a <30-µm-thick symmetrically tapered Ge waveguide. The top flat surface of the waveguide acts as a sensing area, while its ZnSe coated curved bottom helps increase the number of total internal reflections, resulting in enhanced sensitivity, particularly in the middle part of the waveguide.

Broadband IR light from the spectrometer's external beam port is focused onto the entrance end of the waveguide. The output end of the waveguide is butt-coupled against the window of a liquid N₂ cooled HgCdTe immersion detector (MOD-O2S1, Remspec Corp., Sturbridge, MA). The collimated beam exiting the external output port of the spectrometer was simply focused onto the entrance end of the waveguide by using a single offaxis paraboloid mirror with a 19.1-mm focal length. In order to maximize absorption intensity as well as to increase reproducibility, every sample applied in the experiment must be carefully placed in contact with the sensing surface at the thinnest (center) point on the waveguide.

Comparisons to Standard Attenuated Total Refection Measurements. Attenuated total reflection measurements obtained with the tapered Ge waveguides were compared with those obtained by using a commercially available macroscopic ATR accessory (Out-of-Compartment Contact Sampler[®] with 45° Ge Trough Plate Kit, Spectra-Tech, Shelton, CT). For experiments with the commercial ATR accessory, a photodiode HgCdTe detector (Model KMPV11–1-LJ2/239 with 0.785 mm² active area, Kolmar Technologies Inc., Conyers, GA) internal to the spectrometer was utilized. The manufacturer's specifi ed*D** value of this detector at 10 kHz was 3.23×10^{10} cm Hz^{1/2} W⁻¹, compared to $\geq 4 \times 10^{10}$ cm Hz^{1/2} W⁻¹ for the Remspec immersion detector.

For purposes of spectral comparisons between waveguide and commercial ATR accessory, the same experimental parameters were utilized on a Bruker IFS66 FT-IR spectrometer. All IR spectra shown below were acquired by using an 8 cm⁻¹ resolution and a 7800 cm⁻¹ bandwidth. The mirror velocity was 3.164 cm s⁻¹, giving an optical retardation velocity (ORV) of 100 kHz for the HeNe reference beam. The number of coadded scans is mentioned individually in each fi gure. Blackman-Harris 3-Term apodization, Mertz phase correction, and zerofi lling of 2 were set as default acquisition parameters with the gain of 1 under Opus software.

Preparation of Bacteriorhodopsin Film for Light-Dark Experiments. The D96N mutant of bacteriorhodopsin (D96N-bR) used was in the form of purple membranes. This mutant was selected because it has a greatly slowed photocycle when dried from a buffer at an elevated pH, permitting light-dark difference measurements at room temperature. For the ATR-IR experiment, 1 µL of unbuffered D96N-bR suspension with a concentration of 1.3 mg/mL was placed on the central 2-3 mm portion of the waveguide and completely air-dried for ~ 20 min prior to pH adjustment. A droplet of 25 mM TRIS buffer at pH 9.5 was subsequently applied on the thin purple film and dried again. The sample preparation was the same for both the commercial ATR accessory and the 14µm-thick tapered Ge waveguide. Only the total volume of sample solution applied is different in each experiment.

Infrared spectra were collected with either the commercial ATR accessory or the 14-µm-thick tapered Ge waveguide, always using the Bruker IFS66 FT-IR spectrometer. The D96N purple membrane film was illuminated with a standard 150 watt quartz-halogen fi ber-optic illuminator (Model 180, Dolan-Jenner Industries Inc., Lawrence, MA). The illumination and data collection were automatically controlled by rapid-scan time-resolved spectroscopy mode under Opus software. The loop started by taking a background spectrum in dark conditions for 30 s (=192 scans), and then turning on the illuminator to expose the D96N purple membrane fi lm for 20 s. A sample spectrum, representing D96N-bR in the so-called M state, was initiated ~ 1 s after the lamp was turned off and lasted for a duration of 10 s (=64 scans) to complete one loop. The next loop was started after a 1-min delay in order to let the sample relax back to the unphotolyzed state. Averaging many dark/light cycles

was necessary to obtain an adequate S/N ratio in the difference IR spectrum. After finishing all the loops, the alternately collected single-beam spectra of background (i.e., unphotolyzed bR state) and sample (i.e., photolyzed M state) were coadded separately. The final static FT-IR difference spectrum is the ratio of coadded sample and background spectra, converted to absorbance units.

RESULTS AND DISCUSSION

Previous theoretical calculations, based on a planar waveguide extending to infinity in two dimensions and with a finite thickness in the third,² indicate that the highest absorbance sensitivities should be achieved from waveguides with a thickness in the range of $0.5-1 \ \mu m$. Such waveguides are not yet realized due to the brittleness of Ge and other fabrication difficulties.

However, we have now successfully fabricated <30µm-thick symmetrically tapered Ge waveguides, which are the thinnest direct-coupled IREs demonstrated to date. The fabrication and coupling methods differ somewhat from those described in previous publications²⁻⁷ in order to eliminate several diffi culties. First, using a waveguide with symmetrical tapering helps simplify the alignment of the beam and detector when using a direct coupling method, as illustrated in Fig. 1. In particular, the taper yields sufficient throughput energy to come close to saturating a 0.25 mm² HgCdTe detector without requiring an IR microscope as a focusing optical element. The 1mm² ends of the waveguide are large enough to make initial alignment by eye possible. Simultaneously, the taper keeps high the number of reflections in the middle sensing region, where the waveguide is thinnest. In addition, the flat and flush horizontal sensing area of the epoxide-embedded tapered waveguides results in easy sampling and cleaning. Furthermore, completely embedding the Ge strip in the epoxide substrate, instead of cementing just one surface to a quartz substrate as described previously,³⁻⁷ provides more mechanical support for the waveguide. In particular, it reduces the possibility of damage to the waveguide during grinding and polishing.

Variation of Waveguide Thickness. The evanescent wave absorption properties of symmetrically tapered Ge waveguides were investigated after careful alignment, as determined by detected throughput. We fi rst analyzed the throughput as a function of decreasing waveguide thickness. Figure 2 displays several FT-IR single-beam spectra acquired from thin symmetrically tapered Ge waveguides with different thicknesses. As can clearly be seen, the observed throughput diminishes with decreasing thickness, due to cut-off of higher-order modes.²¹⁻²³

The single-beam spectra (Fig. 2) reveal the characteristic frequency cut-offs of Ge at 5200 and 670 cm⁻¹ and demonstrate that light is genuinely guided through the thin Ge layer. The lower-frequency cut-off demonstrates a benefit of using Ge waveguides. In contrast, there is a lack of transparency with silicon (Si) waveguides in the IR region 1500–700 cm⁻¹, where there is rich spectral information for most organic compounds.

In order to investigate the effect of the tapered thickness on waveguide sensitivity, a 1-mm-diameter ($\sim 1 \ \mu L$) water droplet covering the $1 \times 1 \ mm^2$ sensing area was



FIG. 2. FT-IR single-beam spectra of symmetrically tapered Ge waveguides with different thicknesses (i.e., 7, 14, 22, and 27 μ m from lower to upper lines, respectively). All spectra were collected with 1-min scans and under identical conditions. The y-axis scale stands for arbitrary intensity units, which are the same for all plots shown here. As clearly seen, the throughput diminishes with decreasing thickness, with a correlation coefficient of $R^2 = 0.9999$ and 0.9956 for quadratic and linear relationships, respectively (see Inset). The plot fits with the quadratic equation of $y = -0.0002x^2 + 0.0292x - 0.1352$. Inset: Plot of the detected intensity at 1850 cm⁻¹ as a function of the waveguide thickness.

carefully sampled at the same (thinnest) part of each of several waveguides. The resulting absorbance spectra are presented in Fig. 3. It can be seen that the waveguide's sensitivity, in terms of absorbance for a particular smallarea liquid sample, increases as waveguide thickness decreases. This enhanced sensitivity with decreasing thickness is due both to the increase in number of internal reflections per mm of waveguide length and to the increase in the average evanescent wave surface intensity of the transmitted modes.² The inset of Fig. 3 indicates a drastic increase in absorbance when the waveguide thickness is <10 μ m. On the other hand, a very thin waveguide (i.e., <10 μ m) results in distinctly lower total throughput and an increase in spectral noise level. To

improve the spectral quality, a higher number of scans must be used.

Variations in Optical Coupling with Sample. We additionally observed the dependence of absorption intensity on sampling length as shown in Fig. 4. With a planar waveguide, absorbance is expected to increase in direct proportion to sample contact length. With a tapered waveguide, on the other hand, the increase in absorbance as a function of contact length is expected to be sublinear because the average number of reflections per unit length of sample contact decreases as sample is added to regions of the waveguide away from its central minimum. This was observed, as shown in the inset of Fig. 4. Each plot of absorbance at 3400 cm⁻¹ (A_{3400}) as a function of



FIG. 3. ATR-IR spectra of a 1- μ L water droplet, covering an ~1 mm² sensing area, acquired by using symmetrically tapered Ge waveguides with different thicknesses (i.e., 7, 14, 22, and 27 μ m from upper to lower lines, respectively). Original single-beam sample spectra were converted to absorbances by ratioing to the corresponding background spectra shown in Fig. 2 at the same thickness with the same experimental parameters. The spectra with higher absorbances correspond to measurements using thinner waveguides. **Inset:** Plot between the logarithm of the absorption intensity at 3400 cm⁻¹ [log(A_{3400})], due to the O–H stretching vibration, vs. the logarithm of waveguide thickness [log(t)].



FIG. 4. ATR-IR spectra of water droplets of different lengths contacting the sensing surface of the waveguide. (A) Spectra were obtained by using a 7- μ m-thick Ge waveguide, with sampling lengths of 1, 4, 7, 9, and 12 mm from lower to upper lines, respectively; (B) spectra obtained with a 27- μ m-thick waveguide with sampling lengths of 1, 3, 5, 7, 10, 12, 15, 18, and 20 mm from lower to upper lines, respectively. All spectra were taken with 1-min scans. Both data sets over this limited range could easily be fit to quadratic relationships between absorption intensity at 3400 cm⁻¹ (i.e., O-H stretching) and sampling length covered by the water droplet. **Insets**: Plot of the absorption intensity at 3400 cm⁻¹ (A_{3400}) vs. the sampling length (mm) with a constant width of the waveguide (i.e., 1 mm), for different waveguide thicknesses. Data plotted on each graph coincide with the value of A_{3400} appearing on each sub-fi gure.

contact length (l) was fitted to a quadratic relationship. The optimized quadratic coefficients gave $A_{3400} = -0.0009l^2 + 0.0316l + 0.3218$ for the 7-µm-thick waveguide and $A_{3400} = -0.0001l^2 + 0.0073l + 0.0533$ for the 27-µm-thick waveguide. The quadratic fit is good only up to ~2 cm of contact length, after which point the absorbance approaches an asymptotic value monotonically. This observation shows that one advantage of using these <30-µm-thick tapered Ge waveguides is that only molecules in contact with the thinnest part of the waveguide significantly influence the absorbance spectrum.

In an attempt to further improve the waveguide's sensitivity, we explored several additional variations in optical coupling through the waveguide and sample. First, the overall spectral quality obtained from tapered Ge waveguides depends strongly on the quality of the polished sensing surfaces (see Fig. 5). A better optical-finished surface gives not only a higher absorbance due to the better optical contact between sample and waveguide's surface, but the spectral quality is also distinctively improved because of lower scattering losses caused by surface scratches, resulting in substantially greater throughput. The more thoroughly the surface was polished, the lower the absorbance noise (and therefore the limit of detection) of the waveguide.

Substantially greater detected absorption intensity can sometimes be achieved as a consequence of beam blocking of the on-axis waveguide modes (see Fig. 6). This was demonstrated by using an \sim 6-mm-diameter aluminum rod held horizontally transverse to the IR beam where it fi lls an aperture of \sim 30 mm, in order to partially obstruct the beam. Light rays with low propagation angles in the vertical direction are thus blocked by the aluminum rod. At the selected distance from the waveguide, this rod eliminates rays that would enter the waveguide at an angle of $<17^{\circ}$ away from horizontal.

With both waveguide thicknesses investigated, the spectra taken with the centrally blocked beam gave higher absorbance readings than those with an open beam, substantially so in the case of the 22-µm-thick Ge waveguide. The increase in absorption intensity results from selection of only the higher-order modes (i.e., light propagating closer to the normal of the waveguide interface at the points of internal reflection). This is theoretically expected to increase (1) the average number of reflections per unit length; (2) the average evanescent fi eld strength at the sensing area; and (3) the effective average penetration depth into the media beyond the interface. The improvement in absorption intensity is greater with the 22-µm-thick waveguide than with the 14-µm-thick one, probably because the coupling efficiency of the highestorder modes to the detector is always decreased with the thinner waveguide whether or not the central beam is blocked. This observation indicates that blocking even more of the central rays might further increase the absorbance enhancement factor, but would likely give rise to a higher noise level due to the lower total throughput, particularly with thinner waveguides, as clearly seen by comparing the spectral quality in Figs. 6A and 6B.

There are a number of changes in optical coupling that are likely to afford substantial additional improvement in sensitivity, but that we have been unable to investigate



FIG. 5. Effect of the surface quality on absorbance spectral features observed from a 1-cm-diameter water droplet sample. These spectra were collected by using a 100- μ m-thick tapered Ge waveguide with 45° bevel angle at both ends with 1-min scans. Data were obtained by hand-polishing the same waveguide with progressively smaller particle sizes (i.e., 15, 3, and 0.1 μ m from lower to upper lines, respectively).

so far. Ultimately, the most important of these is likely to be optimization of the detector dimensions. Using a HgCdTe detector with a smaller-area rectangular active element whose aspect ratio is more closely matched to the thinnest part of the waveguide is in theory expected to give somewhat better results than we have so far obtained. By limiting ourselves thus far to a square detector, we were faced with the tradeoff between going to the smallest possible detector, leading to an expected noise reduction in proportion to the square-root of the detector area, and keeping the output of the entire width of the waveguide imaged onto the detector, which is necessary for maintaining the largest response.

Applications and Comparisons with a Commercial Ge ATR Accessory. Small Volumes of Volatile Solvent. Figure 7 is the evanescent wave IR spectra of a 1-mmdiameter (=1 μ L) water droplet in contact with the 14- μ m-thick tapered Ge waveguide, compared to that ob-



FIG. 6. Effect of central beam blocking on absorption intensity of 1-mm-diameter (=1 μ L) water droplet investigated by (A) 14- and (B) 22- μ m-thick Ge waveguides with 5-min scans. Blocked-beam spectra were recorded after the beam path was obstructed for rays deviating <17° vertically away from the waveguide axis. In both cases, the spectra exhibit substantially greater absorption intensity enhancement with blocked beam (thick lines) as compared to those with unblocked one (thin lines).



FIG. 7. Comparison of absorption intensities of a 1-mm-diameter water droplet (=1 μ L) observed by the 14- μ m-thick tapered Ge waveguide to those with a commercial macroscopic ATR accessory (*Out-of-Compartment Contact Sampler[®] with 45° Ge Trough Plate Kit, Spectra-Tech). The sample covered the sensing surface with a total area of 1 mm² at the central point. Both spectra represent 5-min scan times.

served with a macroscopic ATR accessory shown in the same fi gure. By comparison, the absorption intensity of the O-H vibration at 3400 cm⁻¹ acquired with the thin tapered waveguide is enhanced by a factor of 37 due to the increase in total internal reflections achieved by the tapering method. Thus, small volumes of volatile solvents such as water that cannot easily be spread out in a thin fi lm on a macroscopic ATR can still be sampled using the waveguide.

Small Quantities of Non-volatile Thin Films. Figure 8 shows spectral results of 85 ng Nujol deposited as a thin fi lm from hexane solution onto the sensing surfaces of either the commercial ATR accessory or the 14- μ m-thick tapered Ge waveguide. The spectra were recorded >1 h after the hexane was visibly evaporated and the fi lm was left exposed to room air. Once again, the 14-

µm-thick tapered Ge waveguide exhibits substantially higher sensitivity than the commercial ATR accessory. The relative absorbance factor observed at 2922 cm⁻¹ was 13.6 for the 85-ng Nujol sample. This clearly demonstrates the advantage of the thin tapered waveguides for measuring sample-limited thin films. With the traditional Ge ATR accessory, the 85 ng sample size gives absorbance signals that are barely above the noise with a 5-min scan time, whereas with our thin tapered waveguides, the S/N ratio of the strongest bands in the absorbance spectrum substantially exceeds 20. Characteristic peaks of Nujol can therefore be easily identified. The estimated detection limit with a 5-min scan time is 85 ng Nujol deposited on the waveguide's sensing surface. This is, of course, substantially higher than the detection limit for a droplet of pure Nujol measured in transmission



FIG. 8. ATR FT-IR spectra of Nujol thin films with different quantities deposited on the sensing surface, i.e., (A) 850 ng and (B) 85 ng. The thick lines represent the results observed by the 14- μ m-thick symmetrically tapered Ge waveguide, while the thin one was collected with the commercial ATR accessory (*Out-of-Compartment Contact Sampler[®] with 45° Ge Trough Plate Kit, Spectra-Tech). All spectra were taken after hexane was completely evaporated and with 5-min scan times.



FIG. 9. (A) ATR FT-IR spectrum of D96N mutant of bacteriorhodopsin (D96N-bR) in the form of a dried fi lm with a total amount of 1.25 pmol (37.5 ng) of protein. The spectrum was acquired with a 14- μ m-thick tapered Ge waveguide with 5-min scan times. The strong bands centered at 1650, 1550, and 1160 cm⁻¹ represent amide(I), amide(II) vibrations, respectively, which are the characteristic spectral features of the peptide backbone. (**B**, **C**) ATR FT-IR difference spectra of D96N-bR using light–dark subtraction at room temperature (25 °C). (**B**) 128 averaged data sets, total measurement time, ~4 h, using the 14- μ m-thick tapered Ge waveguide and 50 pmol protein. (**C**) 64 averaged data sets, total measurement time, ~2 h, using the Out-of-Compartment Contact Sampler[®] with 45° Ge Trough Plate Kit, Spectra-Tech (*) and 15 nmol protein.

mode by a microscope. Nevertheless, for small quantities of poorly soluble samples that can only be deposited uniformly as fi lms under $\sim 1 \ \mu m$ in thickness, the miniature waveguide is likely to afford a superior sampling method.

Light-Dark Difference Spectra of Biological Film Samples. A thin tapered Ge waveguide is able to detect a structural conformation of biological sample covering only 1 mm² on the thinnest middle part of its sensing surface. The D96N mutant of bacteriorhodopsin (D96NbR) was chosen as a model sample for studying the triggered structural change of the protein conformation. As expected, the characteristic spectral features of the peptide backbone (i.e., amide(I), (II), and (III) vibrations) in the purple membrane of D96N-bR can be successfully sensed by using a 14- μ m-thick waveguide with just 1.25 pmol (37.5 ng) of protein sample (see Fig. 9A). Lightdark difference measurements on D96N-bR require somewhat larger samples (50 pmol bR) deposited as a thin film on a 14-µm-thick waveguide. However, this amount is 3000 times less than the 1.5×10^5 pmol of D96N-bR required for similar light-dark difference measurements using a macroscopic Ge ATR accessory. The light-dark difference spectrum obtained with a 14-µmthick waveguide (Fig. 9B) is very similar to that obtained on a much larger sample with a macroscopic ATR accessory (Fig. 9C).

Hair Analysis. Single human hairs represent a challenging type of IR sample that has been investigated with both ATR and FT-IR microscope methods.^{24,25} In our

measurements, a single hair obtained from either an African-West Indian female or a Caucasian male was squeezed against the center of the waveguide sensing area with a ratcheting micrometer in order to obtain reproducible sample contact. Typical results are shown in Fig. 10. Each spectrum observed showed a broad band at 3250 cm^{-1} attributable to water absorption. The predominant absorption bands centered at 1650 and 1550 cm⁻¹ on both single-hair spectra in Fig. 10 arise from amide(I) and amide(II) vibrations, respectively. The amide(I) band is particularly diagnostic for secondary structure and in this case, is at a wavenumber characteristic of the known α -helical structure of hair keratin.²⁴

By comparing the spectra of each individual's hair with pure samples of the respective styling gels each had applied several hours previously, it is clearly seen that even a tiny amount of chemicals deposited on a 2-mm length of hair surface can be detected. In each case, there are several peaks on the measured hair spectrum corresponding to the strongest bands in the spectrum of the corresponding pure styling gel. This indicates the potential utility of the <30- μ m-thick tapered waveguides as a new tool for forensic analysis.

CONCLUSION

Symmetrically tapered Ge waveguides with thicknesses down to $\sim 7 \ \mu m$ have been successfully fabricated and utilized as MIR evanescent wave sensors. Tapering in this



FIG. 10. Baseline-corrected ATR FT-IR spectra of single hairs (upper thin line) taken from two different people, (A) an African-West Indian female, and (B) a Caucasian male. The lower (thick line) spectrum in each sub-figure represents the styling gel applied earlier in the day by each person (i.e., Magnifi cent Hair Food manufactured by Magnifi cent Products Division, Memphis, TN, for donor A, and Aussie Mega Styling Gel[®] manufactured by Redmond Products, Inc. Dist., Stamford, CT, for donor B). Films were dried ~ 1 h prior to spectral measurement. All spectra were acquired with a 14-µm-thick tapered Ge waveguide with 5-min scan times. The strong bands centered at 1650 and 1550 cm⁻¹ on both single hair spectra arise from amide (I) vibrations, respectively

way helps eliminate several problems previously discussed.²⁻⁶ First, a high total throughput can be achieved with an uncomplicated optical alignment procedure and without requiring an IR microscope by using only a single off-axis paraboloid mirror. In addition, a flat horizontal sensing area provides convenience in sampling. It allows for better contact of solid samples such as human hairs, resulting in higher sensitivity and better spectral S/N ratio. As expected, reducing the tapered thickness of the waveguide to $<30 \ \mu m$ produces substantially higher absorbance per unit weight of sample, particularly as compared to a macroscopic Ge ATR accessory. However, this measured increase in absorbance is not yet as great as that predicted for waveguides of this thickness; and it must also be balanced against the significantly lower total throughput that is also not yet close to that predicted to be theoretically achievable.²

Nevertheless, we have demonstrated that these <30µm-thick tapered Ge waveguides have the potential to become a useful technique for at least three types of application that are currently diffi cult with commercial ATR accessories: (1) sub-microliter quantities of volatile liquids; (2) ng-quantity thin-fi lm samples; and (3) coatings and surface layers on small fi brous samples such as individual human hairs. The last application especially indicates the possible utility of these miniature Ge waveguides in nondestructive forensic analysis.

ACKNOWLEDGMENTS

This research was supported by National Science Foundation (NSF) grant MCB-9722887 to M.S.B. and by additional funding from the Keck Center for Molecular Electronics at Syracuse University. Jitraporn Vongsvivut was supported by a fellowship from the Thailand Research

Fund (TRF) via The Royal Golden Jubilee (RGJ) Program 3-C-CU-43-P-1 to Dr. Sanong Ekgasit (Chulalongkorn University, Bangkok, Thailand). We also gratefully acknowledge the assistance of William F. Kutz, K & S Optics, who did most of the work involved in fabricating the waveguides.

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