Angle-Multiplexed Waveguide Resonance of High Sensitivity and Its Application to Nanosecond Dynamics of Molecular Assemblies

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ABSTRACT: We report here the experimental demonstration of a high-performance optical waveguide resonance (WR) platform based on a judicious design of a dielectric/metal stack and a fabrication process that delivers an extraordinarily low-loss optical waveguide over a noble-metal thin film. By using an atomic layer deposition process to grow a dielectric film (Al2O3) of exceptional optical quality and precise thickness over a metal layer (Ag), we have reached a deep and narrow WR that allowed us to experimentally measure a performance of the WR device that is 20 times superior to the conventional surface plasmon resonance sensor. To the best of our knowledge, these results represent the best performance of a WR device reported so far in the literature. In addition, we have created an experimental setup based on diffraction-limited optical components to launch and collect a broad angular spectrum that is able to resolve the sharp angular waveguide resonance at a fast pace. Such configuration has enabled us to reach nanosecond time scale resolution, and we provide here experimental evidence of the fast coupling of the optical signal from a submonolayer of a ruthenium complex adsorbed to the interrogation surface. The high sensitivity and nanosecond detection capability of the WR optical platform demonstrated here are expected to find useful applications for researchers interested in studies of surface-mediated molecular interactions and interfacial phenomena.

Surface plasmon resonance (SPR) is an electronic surface-charge-density wave that propagates along a metal/dielectric interface and can only be excited by p-polarized light (also known as transverse magnetic, TM, polarization) in metals with a negative dielectric constant (e.g., Au, Ag). The surface-charge-density wave creates a strong electromagnetic field at the interface that decays rapidly into the dielectric material in one side and the metal in the other side. Such strong confinement of the electromagnetic field makes SPR devices extremely sensitive to any perturbation in the dielectric constant near the metal/dielectric interface. The SPR reflectance profile (both in the Kretschmann or Otto configurations) against either angle or wavelength shows a strong resonant dip around the plasmon excitation condition. Over the past 2 decades, surface plasmon resonance sensors have been used widely in studying molecular interactions and dynamics. Others have used SPR to understand the behavior of living cells, and SPR is also proving to be a useful tool in controlling food safety issues.

An interesting alternative to the conventional SPR device is a waveguide resonance (WR) structure which is made by overlaying a transparent dielectric film atop of a noble (typically Au or Ag) metal layer. The approach is also known in the literature by optical waveguide spectroscopy or coupled plasmon-waveguide resonance or plasmon-waveguide resonance. In terms of operation, the WR device works similarly to the SPR structure. For example, in the Kretschmann geometry, incident light from a prism is coupled to the waveguide mode at the specific angle that the effective index of the guided mode is matched by the incoming incident light. The guided mode inside the dielectric layer undergoes total internal reflection on one side (the interface between the dielectric film and a semi-infinite medium, typically air or water) and a high reflection on the side of the dielectric/metal interface. Although the majority of the electromagnetic field of the guided mode is inside the transparent dielectric material, a small fraction still penetrates inside the metal film. Therefore, one obtains a waveguide structure that is lossy (due to the metal film) and leaky (due to the presence of the coupling prism). The metal absorption then creates a dip in the reflectance profile when scanned at different angles, as in SPR, but with a much narrower angular width (compared to SPR) due to a longer propagation length as most of the electromagnetic field is inside the highly transparent dielectric film. In contrast to SPR, which is excited only by p-polarized light, both s- and p-polarized modes can be supported by WR devices. Such dual-polarization capability offers possibilities for birefringence studies.

Although those concepts have been reported for some time and several important applications are present in the literature, the experimental implementation of a WR device with a performance superior to SPR has been elusive.
For an unbiased evaluation of the optical performance in devices with different structures, we define a criterion for resolution as the refractive index change, $\Delta n$, that causes a shift in the resonant angle equivalent to one full-width at the half-maximum (fwhm), $\Delta \theta$, of the corresponding angular resonance curve. As illustrated in Figure 1a, the criterion is then expressed mathematically by

$$\Delta n = \frac{\Delta \theta}{\partial \theta/\partial n}$$

(1)

where $\partial \theta/\partial n$ is the rate of change that the resonant angle varies for a specific device. Obviously, the smaller the value of the refractive index change, $\Delta n$, the better is the limit of detection. We understand that is certainly possible to resolve angular shifts that are substantially smaller than the fwhm. However, defining exactly what fraction of the fwhm one can resolve is a controversial matter and irrelevant for this work which focuses on a side-by-side comparison between SPR and WR devices.

The theoretical calculations shown in Figure 1a (and in other figures that appear after) were done using a transfer matrix method\textsuperscript{12} to determine the reflectance versus incident angle of light impinging on a planar stack of thin films at either s- or p-polarization. Our first step in the design process was to define the film stacks that created the best resolution (i.e., the smallest value of $\Delta n$ according eq 1) for SPR and WR devices at the 633 nm wavelength. For the silver-based SPR device (Ag-film of refractive index, $n_{Ag} = 0.09 - 4.10i$, over a BK-7 glass substrate of refractive index, $n_g = 1.51509$, and water superstrate with refractive index, $n_{H2O} = 1.33211$), the optimal thickness of silver was found to be 52 nm. For the alumina-silver-based WR device (Al$_2$O$_3$-film of refractive index, $n_{Al2O3} = 1.63$), the optimal thickness of the alumina and silver films were 146 and 36.5 nm, respectively. The ideal alumina thickness is obtained just above the cutoff value to support the fundamental TE mode and the reflectance curve must show a deep and narrow resonance (i.e., a resonance with a high quality factor). Therefore, in respect to the limit of detection, the WR devices reported so far in the literature are not superior to conventional SPR structures. The experimental demonstration of the ideal WR performance has been hampered by difficulties in creating a high-quality optical waveguide (i.e., a dielectric film that is highly transparent and has a very smooth surface) of precise thickness over a soft noble metal film. Those shortcomings have reduced the quality factor of previous waveguide resonance devices and have degraded the experimental sensitivity when compared to theoretical calculations based on ideal films. In this work we present what, to the best of our knowledge, is the first optimally designed and fabricated WR sensor delivering the best resolution for the technique. By using an atomic layer deposition (ALD) process, we have created a high-quality optical waveguide layer of Al$_2$O$_3$ over a silver film, which allowed us to reach a WR device of improved quality factor whose performance surpasses the behavior of a SPR sensor. In addition, we also report here the implementation of an angle-multiplexed configuration with enough resolution to discriminate the narrow waveguide resonance. Such a setup of high sensitivity and without moving parts was then connected to a fast ICCD camera and enabled us to successfully follow the nanosecond dynamics of surface adsorbed molecules.

![Image](image_url)
is 39 times smaller (therefore better) than its SPR counterpart. For probing analytes adsorbed to the sensor surface, we have modeled a 3-nm adlayer of refractive index 1.46 atop the SPR and WR devices to simulate an immobilized protein monolayer. Again, by using eq 1, we found a SPR resolution of $\Delta n = 0.4863$ and a WR resolution of $\Delta n = 0.2318$, thus a 2-fold improvement of the WR over the SPR device.

The fundamental reason for the superior performance of the optimized WR over the optimized SPR is the much longer propagation length of the former as the majority of the guided mode in the WR device is confined to the dielectric film and thus the optical attenuation is reduced in this case. The longer propagation length of WR translates into a nearly 60 times narrower angular width, $\Delta \theta$, as seen in Figure 1b. On the other hand, the optimized SPR device has a rate of angular change, $\partial \theta / \partial n$, that is approximately twice that of the optimized WR device because its mode profile is more tightly confined to the probing interface. When both effects are taken into consideration, the very narrow width of the waveguide resonance in the numerator prevails over the rate in the denominator of eq 1, leading to a smaller and better resolution for WR devices. The improvement is significant for probing refractive-index changes in the bulk region (39x) but much smaller for probing similar effects in ultrathin adsorbed films (2x).

It is important to notice that if the WR device is not fully optimized, for instance when the dielectric film is too thick or shows substantial propagation losses, the potential benefits of the WR configuration are not materialized. If the dielectric film is too thick (much thicker than the cutoff thickness for the presence of the fundamental TE mode), then the waveguide mode becomes large and the fraction of the electromagnetic field inside the probing region becomes smaller, which mathematically translates into a smaller value for the denominator in eq 1 and thus poor device sensitivity. In the case when the dielectric film shows substantial propagation losses (either by residual absorption or scattering in the dielectric film), then the resonance dip in the reflectance profile does not approach zero and the angular width, which is the numerator term in eq 1, will increase and prevent the WR configuration from reaching its full sensitivity. Therefore, for the full implementation of a superior WR geometry compared to a SPR device, it is crucial to have a well-designed structure and a fabrication process that guarantees a low-loss optical waveguide with the correct thickness atop of the noble metal film. In the sections following, we show the successful realization of those requirements.

■ MATERIALS AND METHODS

Fabrication of WR Device. Microscope slides purchased from VWR were inspected for defects and then thoroughly cleaned by sonication in microdetergent at 60 °C for 10 min followed by scrubbing with cotton swabs for two cycles. Samples were then rinsed in deionized water and placed in a Nano-Strip (Cyantek Corporation) bath at 90 °C for 5 min and rinsed again, followed by soaking in methanol at 60 °C for 3 min. A 36-nm silver film was deposited using electron beam evaporation under approximately $10^{-6}$ Torr vacuum. Without breaking vacuum, an additional 5-nm SiO$_2$ film was deposited above the silver film for protection. The sample was then transferred to an ALD tool, and an alumina (Al$_2$O$_3$) film was deposited using atomic layer deposition. Because of the presence of the 5-nm SiO$_2$ film, the ideal thickness of the alumina film (from our model calculations) became 141 nm and the ALD fabrication process aimed for this value. Because the alumina films were hydrophobic, in a few cases of the experimental work described below, we functionalized the Al$_2$O$_3$ surface with a 1-nm layer of PEG silane (2-[(methoxy(polyethyleneoxy)propyl]trimethoxysilane) by molecular vapor deposition to allow adsorption of fluorescent molecules.

Fluorescent Sample. To demonstrate that our WR device can be used to collect real-time information, the fluorescent decay of Ru(bpy)$_3$(mcbpy-O-Su-ester)(PF$_6$)$_2$ (Ru-complex) was utilized. Benner et al.$^{15}$ reported capturing fluorescence emission with SPR. They observed a peak in intensity at the resonance angle when fluorescent molecules layered on an SPR sensor were excited from the back, proving that the emitted light was coupled into the SPR system.

Ru(bpy)$_3$(mcbpy-O-Su-ester)(PF$_6$)$_2$ was purchased from Sigma Aldrich (excitation wavelength 458 nm, fluorescent emission wavelength 628 nm, lifetime approximately 500 ns). The complex was mixed with anhydrous acetonitrile (99.8%) purchased from Alfa Aesar at a concentration of 0.4928 mM. For the experiment, the stock solution was diluted to a concentration of 8 μM in a buffer of 7 mM PO$_4$ and 1 mM NaCl and a pH of 7.2.

Data Collection Setups. Angular Scanning Configuration. Light from a He–Ne laser was passed through a polarizer and then focused into a 25-μm pinhole by an objective (10x) to eliminate high spatial frequency components. Then, the beam was collimated with an achromatic doublet (effective focal length eff = 200 mm, Thorlabs AC 254-200-A1). An iris (diameter of about 5 mm) was used to control the size of the incident beam. For optical coupling of the surface waves, samples were setup in the Kretschmann configuration with an index-matching fluid used to optically combine the prism-coupler and glass substrate of the sensor. The device was put on top of a high-precision rotation stage for changing the incident angle. Incident and reflected intensities were measured at each angle by using a standard power meter. This experimental setup was used to examine the thickness of each coated layer and to determine the experimental resolution and quality of our devices.

Multiplexed Angular Configuration. For fast measurements in the nanosecond time scale, the conventional scanning angle configuration is impractical. Here, we describe an angle-multiplexed optical system based on diffraction-limited optical components combined with an intensified CCD camera (ICCD PI-MAX 3 from Princeton Instruments) (see Figure 2) that allows for measurements with very sharp angular resolution and

![Figure 2. Schematic of the angular multiplexed setup.](image-url)
a nanosecond time scale. The same set of optical components described above was used for collimating the incident laser beam. However, after the collimating lens, an achromatic doublet (f = 500 mm, Thorlabs AC 254-500-A1) was used to focus the light beam into the device to excite the waveguide resonance. Another achromatic doublet (f = 500 mm, Thorlabs AC 254-500-A1) was used to collect and collimate the reflected light into the ICCD, where each pixel was related to a particular incident angle. By changing the focal length of the achromatic doublets, the angular resolution of each pixel could be adjusted. To calculate the reflectivity, a reference profile was taken. Half of the WR device was coated with the stack of interest (Ag, SiO₂, and Al₂O₃ films), while the other half was coated only with alumina. By totally reflecting the incident light from the alumina-coated area, the intensity of the incident beam at different angles was collected and later used as a reference to calculate the reflectivity of the stack of interest. With this system, fast molecular events on the nanosecond scale can be detected and the entire angular spectrum can be recorded in real-time.

**RESULTS AND DISCUSSIONS**

**Characterization of the WR Device.** The optical properties and thicknesses of the films were obtained by matching angular reflectance data to theoretical calculations. The conventional SPR data, shown in Figure 3a, was collected under a scanning angular interrogation of the silver and silica films deposited on a glass substrate and surrounded by air. Such data allowed us to determine the optical constants of those films. Then, the device was coated with an additional alumina film and new measurements of angular reflectance were collected as before, with the only exception that this time we used s-polarized light. Also, the angular reflectance data as shown in Figure 3b allowed us to precisely obtain the optical constants of the alumina film. It is worth noting that the WR displayed in Figure 3b is not so narrow in this configuration because the stack of films was designed to deliver a sharp resonance when the device is surrounded by water, and this particular measurement was collected in air for the sole purpose of device characterization. However, when the device is then measured in the aqueous environment for which it was specifically designed, the angular resonance is very narrow and deep as data shown in Figure 3c. Above all, the data displayed in Figure 3c represents, to the best of our knowledge, the first experimental demonstration of the strong WR in a configuration that surpasses the SPR performance.

To further validate this point, the sensitivity of the WR device was measured to changes in the bulk refractive index. The resonant angle was measured in water (n = 1.332) and in methanol (n = 1.326) solutions, and three independent measurements were recorded for each liquid. The resolution of the device, as defined by eq 1, was experimentally found to be Δn = 0.0006 ± 0.0002, which is slightly higher than the expected value (Δn = 0.0003), but still demonstrates a sizable improvement (19x) over the theoretical limit of SPR devices as previously calculated (Δn = 0.0117). The small discrepancy between theory and experiment in the WR device could be caused by residual defects in the coated films, small uncertainties in the angular position, or minor contamination of the solutions.

**Characterization of the Multiplexed Angular Setup.** The same sample used in Figure 3c was then tested with the angular multiplexed setup. In order to calculate the reflectance in this configuration, the dark signal D (with He−Ne laser off), the reference signal F (from the unsilvered portion of the sample), and the sample signal W were taken. The reflectance was then calculated using the following expression: R = ((W − D)/(F − D)). The reflectance curve achieved using the angular multiplexed approach was compared to the results from simulation and the scanning angular measurements shown in Figure 3c. As observed in Figure 4, although there is a small amount of ripple on the sides of the resonance, the data clearly resolve the sharp and deep angular resonance. Notice that the reflectance curve, as recorded by the angle-multiplexed setup, has a value larger than one in a few places due to experimental imperfections in the normalization process. However, those experimental artifacts are immaterial for the applications envisioned here.

**Applications.** Surface Adsorption of Ru-Complex. Next, the adsorption of the Ru-complex on the waveguide surface was characterized using the angle-multiplexed setup. Before immersion in the aqueous solution, the samples were coated with a 1-nm layer of PEG silane by molecular vapor deposition to allow for adsorption of the Ru-complex on the sensor surface. Initially a background signal was collected with pure buffer solution inside the flowcell surrounding the waveguide device described in the previous section. Then, the Ru-complex solution was slowly injected into the flowcell and the time evolution of the adsorption process was followed by observing
When the equilibrium the angle of resonance had increased by 0.021°, data shows that after the adsorption process had reached injection of the Ru-complex, and after desorption of loosely bound molecules. By comparing to the angular spectrum of the pure buffer solution, the dashed line is 4 min after injecting the Ru-complex solution, and another reflectance profile was acquired. Figure 5 shows the angular reflectance traces of the angular reflectance profile of a He−Ne laser at different times. When no further shift was observed in the resonant angle (about 6 min after Ru solution injection), an aliquot of buffer solution was injected into the flowcell to remove nonadsorbed Ru-complex molecules and another reflectance profile was acquired. Figure 5 shows the angular reflectance traces of the Ru-complex solution, and the dashed line is 4 min after flushing the flowcell with buffer solution.

Figure 4. Experimental results from the angular-multiplexed setup (dashed line) with the ICCD and no moving parts compared to the results already shown in Figure 3c of the scanning-angle measurements (diamonds) and modeling calculations (solid line). The sample is the same as the one characterized in Figure 3c.

Figure 6. Adsorption of the Ru-complex on the waveguide surface. The solid line is the angular reflectance spectrum taken with the device under buffer solution, the dotted curve is 6 min after injection of the Ru-complex solution, and the dashed line is 4 min after flushing the flowcell with buffer solution.

The work described here is expected to provide an improved capability of the angular-multiplexed waveguide resonance setup. We have shown here that WR devices need to be properly designed and fabricated in order to materialize a performance improvement over SPR sensors. By using atomic layer deposition to create a high-quality optical waveguide over a silver film, we have experimentally achieved a WR device of outstanding performance and demonstrated experimentally a limit of detection that is 20 times better than the theoretical value prescribed by SPR. We have also demonstrated that our WR platform, when combined with an aberration-corrected angle-multiplexed setup and a fast array detector, is capable of detecting fast molecular dynamics at the nanosecond time scale. The work described here is expected to provide an improved ground for the practitioners of the WR technology, which is

Nanosecond Time-Resolved WR Detection. The emission of the Ru-complex adsorbed onto the waveguide surface was then used to demonstrate the nanosecond time resolution capability of the angular-multiplexed waveguide resonance setup. A He−Ne laser was first used for alignment of the optical path and angular calibration of the ICCD camera. After the adsorbed film and buffer solution had stabilized, the He−Ne was turned off and a pulsed laser was turned on to excite the surface-adsorbed Ru-complex molecules. The tunable pulsed laser was centered at 458 nm (pulse duration approximately 5 ns), and a 550-nm long-wave pass filter (Thor Laboratories) was placed in front of the ICCD to eliminate any stray light from the pulsed laser. The angular spectrum from the WR device was taken at 10, 120, 230, 340, 450, and 1000 ns time delays after the laser pulses set at a repetition rate of 20 Hz. The ICCD measurement parameters were set to 100 ns gate width, 15K accumulations, and gain of 50.

Upon excitation, the adsorbed Ru complex molecules radiate in every direction and the light that is partially transmitted through the device and prism was captured by the detection unit as schematically shown in Figure 2. However, light emitted at the resonant angle was substantially coupled into the waveguide mode which created a dip in the trace of the angular spectrum, as shown in Figure 6a. With increasing time delays, the fluorescence emission intensity surrounding this dip is then decreased, as shown in Figure 6a. This conclusion is supported by the fact that the angular location of the dip is the same as the resonant angle shown in Figure 5. In addition, these phenomena were not observed in control experiments in which pure buffer solution (no Ru film present) was excited with the pulsed laser (data not shown here).

Next, fluorescence detection data were taken with a polarizer to filter either p- or s-polarizations from the emitted light. The intensity of the s-polarized emission is shown in Figure 6b for varying time delays. There was a clear dip feature at the resonant angle for the s-polarization, which can be attributed to fluorescent emission coupling into the waveguide mode. The height of the dips for different time delays was used to calculate the fluorescence lifetime of the Ru-complex film; results are shown in Figure 6c with an exponential fitting. The fitting result gave 434.8 ns for the emission lifetime, which is consistent with the expected value of 500 ns (Sigma Aldrich).

Then p-polarized light outputs at different time delays were used as a control experiment (data not shown). As expected, no strong coupling occurred in these measurements because the waveguide layer is too thin to support a p-polarized guided mode. Also, this information further validates our previous description.

CONCLUSIONS

We have shown here that WR devices need to be properly designed and fabricated in order to materialize a performance improvement over SPR sensors. By using atomic layer deposition to create a high-quality optical waveguide over a silver film, we have experimentally achieved a WR device of outstanding performance and demonstrated experimentally a limit of detection that is 20 times better than the theoretical value prescribed by SPR. We have also demonstrated that our WR platform, when combined with an aberration-corrected angle-multiplexed setup and a fast array detector, is capable of detecting fast molecular dynamics at the nanosecond time scale. The work described here is expected to provide an improved ground for the practitioners of the WR technology, which is
already proving to be an important tool\textsuperscript{10b} in studies of structure and dynamics of biomolecular films and surface-adsorption phenomena.

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\section*{Notes}
The authors declare no competing financial interest.

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