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Novel, high-quality surface plasmon resonance microscopy

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Abstract

A surface plasmon resonance microscope capable of high-quality speckle-free imaging has been designed that uses a laser as a source. An inexpensive acoustic transducer is used to reduce speckle and other image artifacts arising from the use of illumination from an inexpensive laser pointer. The microscope is described and operation of the system demonstrated.

Keywords

Surface plasmon resonance microscopy; Speckle-free imaging; Laser pointer; Cost-effective imaging

1. Introduction

Surface plasmon resonance (SPR) is based on the existence of charge density oscillations at the interface between two media with dielectric constants of different signs, for example between a noble metal and glass. These charge density waves can be induced when the wavevectors of incoming photons match the wavevectors of the surface plasmons for a given frequency. Surface plasmons are induced by light that is TM-polarized, and wavevector matching is characterized by a drop in the photon flux reflected from the interface. Fields associated with surface plasmons extend into the media surrounding the interface, decay exponentially, and are sensitive to changes in the media around them. This is manifested as a reflectivity change as a function of near-surface refractive indices (RI) [1]. SPR is widely used as an analytical tool in biological and chemical sciences [2–8]. Surface plasmon resonance microscopy (SPRM), as introduced by Rothenhausler and Knoll [9], uses the excitation of surface plasmons to interrogate simultaneously the near-surface refractive index (RI) at multiple sites at a sample surface.

SPR microscopes using lasers can suffer from diminished image quality due to speckle artifacts [10,11]. Many SPR microscopy systems, including one from our laboratory [12], have used incandescent lamps for light sources, as these non-coherent sources do not generate speckle artifacts. However, disadvantages include low efficiency, high heat output, and the need for expensive filters and complex optics. LED-based sources are becoming viable, yet can have a large spectral width, can be difficult to collimate, and may require diffusers and polarizers [13]. Diode-based laser sources can provide a linearly polarized output, narrow bandwidth and a collimated beam in a highly compact low-cost package; we

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have developed a novel and simple scheme using a simple diffuser that dramatically improves laser-based SPRM image quality.

2. Experimental

The schematic of one new microscopy system developed in our laboratory is shown in Fig. 1, in the Kretschmann configuration [14]. The light source used in this study was an inexpensive diode pumped solid-state laser pointer with the output centered at 593 nm available commercially (Laserglow, Ontario, Canada). The average output power was approximately 1.17 mW, as measured by a power meter (Coherent model 212, Santa Clara, CA), and was linearly polarized. The laser pointer was mounted on a four axis and rotation stage (Newport Corporation, Irvine, CA) using a custom mount. This arrangement allows control over laser direction and polarization orientation. The laser could be powered by two AA batteries, but to achieve greater stability and experimental convenience, a low-cost 3V adaptor (0.8 A, Radio Shack, Fort Worth, TX) was used.

In addition to speckle [15], image artifacts can also be produced if the laser output consists of multiple modes creating unwanted spatial intensity gradients. To minimize these drawbacks, the system described here incorporated an inexpensive acoustic transducer-driven oscillating diffuser. Light from the source was reflected from the surface of the diffuser, which consisted of a thin scattering reflective membrane composed of aluminum foil with the diffuse or “dull” side as the reflective surface, mounted over an acoustic transducer (ACS43, Altec Lansing, Milford, PA). The use of a rough, scattering surface may promote speckle formation due to variations in surface height. However, the diffuse reflection allows the use of laser sources not emitting solely in the TEM₀₀ mode (as commonly found in inexpensive diode lasers), by reducing the impact of the specular component of the reflected wave. The aluminum foil was stretched and supported on the speaker frame. Upon activation, sound from the transducer caused the membrane to oscillate sympathetically, changing the path length of light and causing changes in the illumination pattern.

Typically the field amplitudes of the laser radiation is randomly distributed and follows a negative exponential distribution $p(I) = e^{-I/\bar{I}}$, where $\bar{I}$ is the average intensity of the image [16]. The conventional measure of speckle in an image is the speckle contrast K , defined as [17]

$$K = \frac{\sqrt{\sum_{i=1}^N (I_i - \bar{I})^2 / (N - 1)}}{\bar{I}}$$

where I_i is the pixel intensity of the i th pixel and N is the number of pixels. The coherence factor is not taken into account in our calculations of speckle contrast [17,18]. Changes in the path length of light create temporal variations in the spatial distribution of light striking the detector with a time constant dependent upon the velocity of the diffuser. The normalized electric field intensity autocorrelation function is defined as [18]

$$C(t) = \frac{\langle I(0)I(t) \rangle^2}{\langle I \rangle}$$

The average speckle contrast over a time interval T can then be shown to be decreased by [17,18]

$$K = \sqrt{\frac{1}{T} \int_0^T \left(2 - \frac{2t}{T} \right) (C(t) - 1) dt}$$

Thus, given a sufficiently long camera integration time relative to the period of membrane oscillation, temporal averaging of the speckle pattern takes place [15]. Freeware software (Sigjenny, Natch Corporation UK, <http://www.natch.co.uk/>) was used drive the acoustic transducer. After being diffusely reflected, the divergent beam was collimated by a 5 cm focal length convex lens (Newport Corporation, Irvine, CA) and directed to an aperture (Newport Corporation, Irvine, CA) to control the beam diameter. A Kretschmann configuration was used in this microscope using an isosceles BK-7 prism mounted on a rotational stage (Melles-Griot, Carlsbad, CA) to couple light into the gold film. After reflecting off the prism (or gold film to which it was coupled), the light passed through a convex imaging lens and a 0.5 ND filter (Edmund Optics, Barrington, NJ), and was focused onto a low-cost CCD camera (PL-A662, Pixelink, Ottawa, Canada) with a pixel pitch of 6 μm and sensor size of 10.16 mm $\times$ 7.63 mm (1280 $\times$ 1024). The pixel intensity range of the camera is 8 bits in each of the R, G and B channel. No contrast enhancement schemes were used and 0,0,0 corresponds to black and 255,255,255 corresponds to white in the images. An integration time of 50 ms was used in our study. All images shown in this study are raw captures without any processing or rescaling of intensity ranges. Differences in the apparent reflectivity of certain regions between different images can be caused by fluctuation of the laser power output as a function of time. Quantitative reflectivity measurements were obtained by normalizing the pixel intensity values in the region of interest, to the pixel intensity value of a static region on the sensor. The reflectivity of the adhesive was determined by measurement of the adhesive under transverse electric (TE) polarized illumination. This technique provided stable reflectivity values over the time course of our experiments (15 min).

For calculation of speckle contrast, the images were converted to grayscale using Matlab (rgb2gray). Dimensions of the SPR sensor surface are 17 mm $\times$ 11 mm. To obtain the best image, the optics were arranged to satisfy the Scheimpflug condition [19]. To determine the optimal frequency used for the oscillating diffuser we compared contrast values over a range of frequencies at a fixed transducer intensity. The value of 100 Hz provided the lowest contrast in the system (data not shown). To determine the optimal intensity we set the frequency to 100 Hz and the compared speckle contrast values over a range on transducer intensities. In general, we determined that increasing the intensity resulted in lowered speckle contrast; however if the transducer intensity is set too high rupturing of the aluminum sheet can take place. We therefore conducted our experiments at the greatest

intensity possible without damaging the diffuser. Although the mechanical coupling of the diffuser to the transducer was not explored in detail, the power consumption of the transducer at this setting was 0.45 W determined by measuring the driving voltage and speaker impedance.

3. Results and discussion

With the transducer active, it was possible to nearly eliminate artifacts in the image as demonstrated in Fig. 2. Variables affecting speckle size include the wavelength of the light used, distance and surface height variations of the scattering object, apertures and optical magnification [16]. To quantify the efficacy of the vibrating diffuser light was passed through the prism and totally internally reflected into the camera without a gold film in place. The image size was 1280×1024 ; an 18×18 pixel region was chosen for image analysis and a linear curve fit was applied across the selected region to minimize the impact of nonuniform illumination. Measurements of speckle contrast were taken with the acoustic transducer active and disabled. Additionally, since the rough surface of the diffuser can increase the presence of speckle, we set up an alternative configuration using a beam expander to increase and collimate the laser output. This non-diffuser configuration is typical of a more conventional SPR microscopy arrangement, and provides a baseline for the efficacy of our approach. With the transducer disabled, average speckle contrast was measured to be 0.30 ($\sigma = 0.002$, $N = 5$), the non-diffuser configuration yielded a value of 0.11 ($\sigma = 0.002$, $N = 5$) and the active transducer gave a value of 0.01 ($\sigma = 0.002$, $N = 5$), indicating an order of magnitude improvement using the oscillating diffuser.

To test the system, a clean glass slide (Fisher Scientific, Pittsburgh, PA) was coated with a 1 nm layer of Cr (for adhesion), followed by a 45 nm layer of Au using an electron-beam evaporator (Washington Technology Center, Seattle, WA). The Au surface was treated for 30 min using an UV-ozone cleaner (UV-PSD, Novascan, Ames, IA). Four 5 μ L spots of Texas-Red labeled bovine serum albumin (BSA), (A23017, Invitrogen, Carlsbad, CA) at a concentration of 1 mg/mL in PBS were deposited upon the Au-coated slide, forming a test pattern. Incubation for 45 min took place in a sealed Petri dish with a damp paper towel (to avoid solvent loss by evaporation), after which the remaining droplet was pipetted away. A Mylar flowcell was then constructed [5] upon the Au-coated slide and rinsed with PBS. The slide was then imaged through the SPR microscope with the protein sample under PBS; the resulting image is shown in Fig. 3a. To verify that it was indeed the patterned BSA viewed with the SPR microscope, the slide was then viewed under a conventional epifluorescence microscope (Zeiss ICM 405, 2 $\times$ objective, TRITC filter set, 5 s integration time). In Fig. 3b shows the correlation between the SPR microscope image and the epifluorescent image. Note that the SPR image is compressed in the horizontal axis due to the high incident angle of the laser. Similarly, the sequence in Fig. 4 shows images of patterned BSA upon a gold substrate, and illustrates the change in resonance condition as the angle of illumination is changed.

To quantify the system response due to changes in the refractive index over the surface, calibration using NaCl solutions of different concentrations and refractive indices was performed [20]. The refractive index of these solutions was measured using a refractometer

(J157 Rudolph Research, Hackettstown, NJ). An Au-coated slide was cleaned using the procedure above, and coated with a layer of poly-ethylene-glycol-thiol in ethanol (5 mM). The ethanol was allowed to evaporate and a Mylar flowcell was constructed through which the NaCl solutions were passed. Quantitation of the reflectivity took place by normalizing the pixel intensity in the flowcell region by the pixel intensity in the adhesive region. The instrument response in terms of reflectivity versus refractive index is shown in Fig. 5, where the linear response can be clearly seen.

4. Summary

An inexpensive laser pointer based SPRM system that dramatically reduces speckle producing high quality images has been demonstrated. To the best of our knowledge, this technique of time-averaging using an oscillating membrane to reduce laser speckle and the impact of multi-mode laser illumination has not been implemented previously in an SPR microscope, although it has been used in other systems [21–24]. We successfully reduced the speckle contrast by an order of magnitude using the oscillating diffuser. The SPRM system described here could form the diagnostic element for parallel detection of multiple analytes in lab-on-a-chip applications. We are currently working on the use of a more stable source, determining the system LOD and sensitivity characteristics, and engaging in immunoassay development utilizing this technology.

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Fig. 1.
Top view schematic of the SPR microscopy system with the sample oriented horizontally.
The image is not to scale.

Fig. 2.

Images that illustrate the efficacy of the oscillating membrane. Images were not manipulated for display purposes: (a) illustrates an image from the non-diffuser configuration: image artifacts are clearly seen, (b) illustrates the same sample in our new configuration with the transducer disabled: image artifacts have increased and (c) is the same image with the transducer active, in which the pattern can be seen quite clearly. Note the compression of the image in the x -axis due to the high incident angle.

Fig. 3.

Confirmation of the functionality of the SPR microscope by correlation of images of a sample using SPR and fluorescence microscopes. All images are raw, and intensities have not been modified for display (a) surface plasmon resonance microscopy image of protein spots on the Au surface formed as described in the text. Compression in the x -axis is due to the high incidence and reflection angle. The edge of the channel is also visible in the top right, exhibiting high reflectivity. (b) Fluorescence image of the same spotted Au surface. The correlation between the two images is shown by the red lines.

Fig. 4.

The effect of scanning the angle of incidence through the optimal resonance condition for image contrast and quantification. Image intensities were not manipulated for display. The change in the image at 76.8° is due to an adjustment of the optics to prevent the beam from shifting off the CCD camera. Angles were measured using the precision rotation mount upon which the prism is mounted.

Fig. 5.

Illustrating the change in reflectivity as a function of refractive index. To compensate for laser power output variations, normalization of the intensity in the region of interest in the flowcell took place against the static adhesive regions surrounding the flowcell. The different refractive indices were obtained by flowing NaCl solutions through the sample flowcell.