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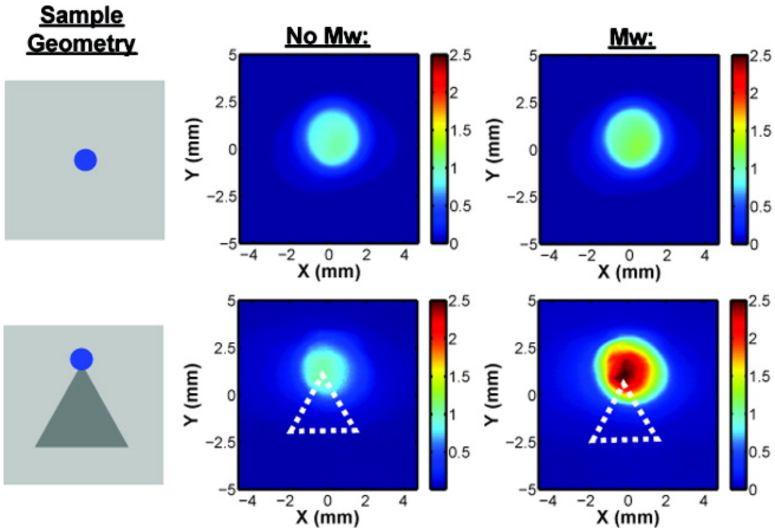

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Microwave-Triggered Surface Plasmon Coupled Chemiluminescence

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Surface plasmon spectroscopy (SPS) is commonly implemented as an analytical tool to characterize thin kinetic processes and binding reactions at interfaces.[1] Due to the limited sensitivity of SPS for the detection of dilute analytes of low molecular mass, surface plasmon coupled fluorescence (SPCF) or, alternatively, surface plasmon coupled emission (SPCE) has been proposed as an alternative means to improve the sensitivity of the technique.[2,3] While SPCF was initially implemented to detect isotropic fluorescence emission,[2] SPCE demonstrated that the emission from randomly oriented fluorophores close to metal surfaces was polarized and directional through the back of the thin metal film.[3] As a result, SPCE has facilitated improved detection of chromophores bound to thin metal continuous films.[3]

In addition to directional and polarized fluorescence emission, our group has also reported surface plasmon coupled phosphorescence (SPCP)[4] and surface plasmon coupled chemiluminescence (SPCC).[5,6] As a result, surface plasmon coupled phenomena can be implemented to improve the detectability of biomolecular interactions at surfaces from weakly emitting species. In recent reports, we also introduced a new technology, microwave-triggered metal-enhanced chemiluminescence (MT-MEC),[7] to increase the detectability of surface-bound HRP enzyme. With MT-MEC, short microwave pulses triggered increased photon flux from HRP-catalyzed chemiluminescent reactions.[8,9] More recently, we implemented planar aluminum structures to focus microwave fields in a conventional microwave cavity and, subsequently, locally "trigger" chemiluminescent reactions.

Combining the advantages of SPCC, MT-MEC, and microwave-focused chemiluminescence (MFC) technologies, we now introduce a new platform technology, microwave-triggered surface plasmon coupled chemiluminescence (MT-SPCC). With this new technique, we present a proof-in-principle method that combines the benefits of directional and polarized plasmon coupled emission with a microwave technology that induces locally "triggered" chemiluminescence emission.[10] As a result, this technique may be implemented to further facilitate high sensitivity detection for chemiluminescent based surface assays, such as immunoassays.[9,11,12] In this paper, the proof-of-principle of this new technique, microwave-triggered surface plasmon coupled chemiluminescence (MT-SPCC), is presented.

MT-SPCC experiments were performed using green emission chemiluminescent reagents as previously described.[7] Surface plasmon coupled chemiluminescence was collected using an optical scheme shown in Figure 1. Briefly, a small (approximately 1 in.) opening was created in the base of a conventional 2.45 GHz microwave cavity, and an optical configuration was designed to maximize the collection efficiency of the directional chemiluminescence emission. Pulsed SPCC intensities can be detected with a high sensitivity CCD (Retiga-SRV, QImaging, Inc.) or a fiber coupled to a spectrophotometer to collect time-dependent emission spectra with a time resolution of 100 ms (Figure 1, bottom right).

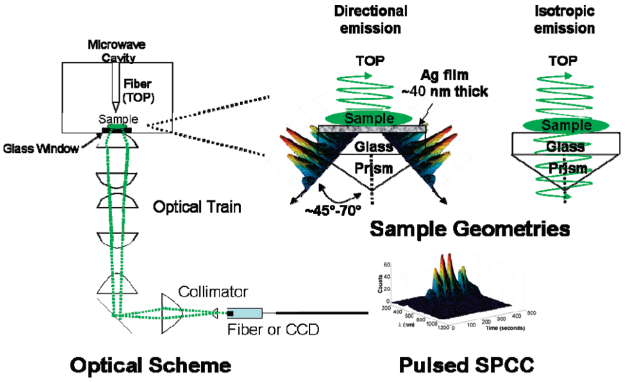

Figure 1. Microwave-triggered surface plasmon coupled chemiluminescence (MT-SPCC) optical scheme (left). Sample geometry for MT-SPCC experiments (top right) and typical MT-SPCC result (bottom right).

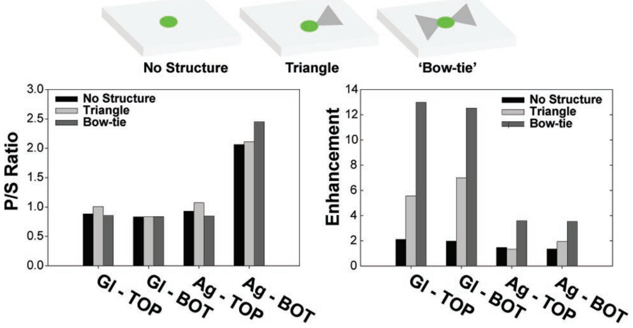

Figure 2. Triangle and bow-tie 2.5 mm equilateral structures 50 nm thick were vapor deposited on Ag continuous films 40 nm thick and glass slides (BOT, signal detection at bottom; TOP signal detection at top). Polarization ratios for MT-SPCC sample geometries (left). Intensity enhancement factor (Mw/no Mw) for MT-SPCC sample geometries (right).

Sample geometries were arranged as shown in Figure 1. In order to demonstrate the feasibility of localized MT-SPCC, 2.5 mm equilateral triangles, Ag continuous films 50 nm thick were vapor deposited on glass substrates with and without continuous silver films approximately 40 nm thick (Figure 2, top middle). The "bow-tie" configuration was achieved by aligning the apex of an inverted 2.5 mm triangle with the tip of the mirror triangle, such that the tips are separated by a gap distance of 1 mm (Figure 2, top right). Imaging chambers were affixed to the center of 5 × 5 mm substrates and proximal to apexes of substrates modified with Ag triangles 50 nm thick. Chambers were subsequently filled with 20 μL of green chemiluminescence solution. The substrates were affixed to a glass prism using index matching immersion oil. Samples were positioned relative to the optical scheme at the base of the microwave to maximize the collection efficiency of the broad angular distribution (∼47−70°) inherent in plasmon coupled directional chemiluminescence.[5,6]

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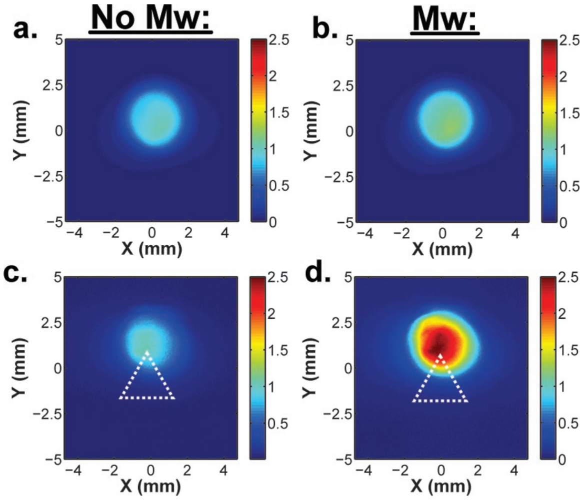

Figure 3. Normalized CCD images of SPCC from (a,b) Ag continuous films 40 nm thick and (c,d) Ag continuous films 40 nm thick modified with 2.5 mm Ag triangle structures 50 nm thick, before (no Mw) and during (Mw) application of a short microwave pulse.

Using the fiber detection scheme and spectrophotometer, we measured the s- and p-polarized intensity for free space (top) and plasmon coupled (bottom) chemiluminescence emission using a sheet polarizer. For SPCC measurements, the sheet polarizer was placed beneath the glass prism and above the intermediate collection optics to ensure polarization properties of chemiluminescence are not perturbed by the intermediate optics. In order to determine the polarization ratios of pulsed chemiluminescence, the p:s ratio was determined from the maximum chemiluminescent p- and s-polarized emissions that were recorded during the application of the microwave pulse (Figure 2, left). We observed that the p:s polarization ratios for the chemiluminescence emission from the Ag continuous films substrates with and without vapor deposited triangle and bow-tie structures are greater than 2 both before (data not shown) and during the application of a short microwave pulse (Figure 2, left). This result is consistent with our previous findings that some s-polarized emission is evident for slowly decaying luminophores, such as chemiluminescent and phosphorescent species.[4−6] In addition, the p:s polarization ratio calculated for all SPCC substrate geometries is approximately equivalent to previously reported p:s ratios for chemiluminescent species.[5,6]

The *enhancement factor* for each of the sample geometries is determined from the ratio of the maximum intensity during the application of the pulse (Mw) and the intensity of steady-state chemiluminescence emission (no Mw). Due to intense field distributions and subsequent heating of solutions in proximity to the tips of the triangle and in the gaps of the bow-tie Ag structures deposited on glass substrates, we observed 6-fold and greater than 12-fold *enhancement factors* for the chemiluminescent reactions on these substrates, respectively.[10] On plain glass substrates, we observed approximately 2-fold enhancements that arise from the absorption of microwaves and subsequent heating of the chemiluminescent solutions.

Upon exposure to low power microwaves, we observed almost no increase in free space chemiluminescence and SPCC on continuous Ag films. As we previously reported, continuous metal surfaces are perfect conductors and experience little ohmic heating in the presence of the microwave fields. As a result, we observe little increase in the chemiluminescent signatures on the continuous Ag films. On the other hand, in the presence of deposited Ag triangle structures, we can increase the SPCC or MT-SPCC intensity. Since the triangle structures are raised above the metal surface, the electric fields build up at the tip or gaps of the triangle or bow-tie structures, respectively, which results in subsequent heating of the proximal solution on the surface.[10] As a result, we observed an approximately 2.5- and 4-fold *enhancement factor* in free space and SPCC emission.

Using a CCD camera, we also captured the *evolution* of the directional chemiluminescent intensity during the application of a short (5 s) microwave pulse. The relative intensity increase is calculated as the ratio of the maximum microwave emission and the pre-microwave emission. We created pseudo-color images to demonstrate the localized SPCC enhancement ratio before and during microwave exposure from continuous Ag film substrates (Figure 3a,b) and continuous Ag film substrates modified with a continuous film triangle (Figure 3c,d).

With these results, we demonstrate that we can combine plasmon coupled chemiluminescence with on-demand chemiluminescence using localized microwave fields. Further studies to optimize structure geometries are underway. When combined with immunoassays, we believe that this platform technology will facilitate increased sensitivity of these surface assay applications. In addition, MT-SPCC surface geometries can be further optimized to maximize field enhancements and subsequent chemiluminescence enhancements. We subsequently believe that MT-SPCC will lead to improved, cost-effective, and portable devices for a host of small molecule sensing applications.

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Supporting Information Available: Materials and methods are supplied. This material is available free of charge via the Internet at http://pubs.acs.org.

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