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Metal-Enhanced Chemiluminescence: Advanced Chemiluminescence Concepts for the 21st Century

Kadir Aslan and Chris D. Geddes*

The Institute of Fluorescence, University of Maryland Biotechnology Institute, 701 East Pratt Street, Baltimore, MD, USA, 21202

Abstract

Chemiluminescent based detection is entrenched throughout the Biosciences today, such as in blotting, analyte and protein quantification and detection. While the biological applications of chemiluminescence are forever growing, the underlying principles of using a probe, an oxidizer and a catalyst (biological, organic or inorganic) have remained mostly unchanged for decades. Subsequently, chemiluminescence based detection is fundamentally limited by the classical photochemical properties of reaction yield, quantum yield, etc. However, for the last 5 years, a new technology has emerged which looks set to fundamentally change the way we both think about and use chemiluminescence today. Metal surface plasmons can amplify chemiluminescence signatures, while low-power microwaves can complete reactions within seconds. In addition, thin metal films, can convert spatially isotropic chemiluminescence into directional emission. In this timely forward looking *tutorial review*, we survey what could well be the next-generation chemiluminescent based technologies.

1. Chemiluminescence

Chemiluminescence is a useful analytical tool for the detection and quantification of a wide variety of biological materials such as cells,¹ microorganisms,^{2, 3} proteins,⁴ DNA⁵, RNA^{6, 7} and also other analytes.^{8, 9} For detailed information on the specific applications of chemiluminescence, the reader is referred to the references given here.^{1–9} The usefulness of chemiluminescence is due to its simplicity and the absence of unwanted background luminescence. In chemiluminescence-based detection, no excitation source and no optical filters are required as compared to other optical techniques such as fluorescence and phosphorescence spectroscopy.¹⁰ Chemiluminescence emission is generated by photochemical reactions and is directly related to the concentration of the reactants. The chemical reactions involve the oxidation of an organic dye by a strong oxidizing agent in the presence of a catalyst (chemical or biological). The most commonly used dyes are luminol and acridan,¹¹ which are not luminescent in the ground state (before an oxidation reaction, Figure 1A). The oxidation of luminol or acridan with hydrogen peroxide (oxidizing agent) in the presence of a catalyst results in the conversion of the ground state of luminol or acridan into an activated state (chemically induced electronic excited states). A strong blue emission (at 450 nm wavelength) can be observed as a result of the decay of the excited states back to the ground state. Chemiluminescence solution emission can last from seconds to hours depending on the quantity of reacting species (Figure 1B). The versatility and simplicity of chemiluminescence has also led to household products/toys such as “glow sticks”, which typically employ organic dyes which can emit three primary colors: red, green and blue (Figure 1C). On the other hand, while chemiluminescence is a versatile tool several factors limit the

*Corresponding Author geddes@umbi.umd.edu.

efficacy of the chemiluminescence based detection in the Biosciences: 1) the quantum efficiency of the organic dye, which results in poor signal-to-noise ratios at low analyte concentration 2) long time before total decay of the emission (i.e. traditional chemiluminescence slow glow). In this regard, an increased chemiluminescence yield and accelerated chemiluminescence reactions would clearly be beneficial for the sensitivity and rapidity (when needed) of chemiluminescence-based bioassays and other technologies. Currently, additional chemical compounds based on the phenyl group or even inorganic ions, are employed to increase weak chemiluminescence emission.^{11–14}

To this end, our research laboratory at the University of Maryland has both introduced and demonstrated several new chemiluminescence concepts, which encompass the interactions of chemically induced excited states and metal nanoparticles, as well as metal thin films, in combination with electromagnetic energy (microwaves), namely, metal-enhanced chemiluminescence, microwave-triggered metal-enhanced chemiluminescence, and surface plasmon coupled chemiluminescence. In this review, we summarize these new timely concepts which we have shown to significantly improve the sensitivity and the rapidity of the chemiluminescence technology.

2. Advanced Chemiluminescence Concepts: I. Metal-Enhanced Chemiluminescence

Metal-Enhanced Chemiluminescence (MEC), a phenomenon first introduced in 2006 by our research laboratory,^{15, 16} describes the near-field interactions of chemically induced excited states with surface plasmons in metal nanoparticles. In MEC, the excited state energy of chemiluminescent species in close-proximity (< 100 nm) to metal nanoparticles is partially transferred (coupling) to surface plasmons and is subsequently emitted with the identical spectral characteristics of the chemiluminescent species in addition to the uncoupled unperturbed free-space emission (Figure 2A). This results in a significant increase in emission intensity, which is attributed to a plasmon-based luminescent enhancement.^{15, 16} It is pertinent to note that surface plasmons are the free electrons in metals those collectively oscillate at frequencies similar to light. Subsequently, light at visible spectral range can induce the surface plasmons in most metals. Since the wavelength of luminescence emission and the surface plasmons overlap, the excited state energy of chemiluminescent species can be coupled to surface plasmons.

Historically, the observation of the MEC phenomenon can be traced to our interpretation of another closely related phenomenon, Metal-Enhanced Fluorescence (MEF).¹⁵ In this regard, we provide a brief description of the MEF phenomenon. For further information on MEF the reader is referred to several other original and review articles found in the literature.^{17–20} In MEF, fluorescent species are excited with an external light source and the energy from the electronically excited states (fluorescence emission) is partially transferred (coupled) to surface plasmons in metals. This process is often referred to as an induced mirror dipole in the metal.²¹ The coupled emission is subsequently emitted by the metal-fluorophore “unified system” with the identical spectral characteristics of the fluorophore (Figure 2B).²² As a result of the plasmon-coupling, two distinct observations can be made in MEF 1) an increase fluorescence emission and 2) a decrease in the lifetime of the fluorophore, where the coupled quanta follows the radiative lifetime of the plasmons themselves.²¹

Although historically, the initial choice of metal for MEC (and MEF) is usually silver, other metals such as chromium,²³ zinc,²² copper,²⁴ gold²⁵ and aluminum²⁶ can also be used. It is important to note that the MEC phenomenon was first demonstrated using Silver Island Films (SIFs) deposited onto a glass microscope slide.¹⁵ Typically, several methods are employed for the characterization of the physical and optical properties of SIFs and other metal nanoparticle-

deposited planar surfaces. Atomic Force Microscopy (AFM) allows the collection of information on the physical features of the metals on a surface. A typical AFM image of SIFs deposited onto a glass microscope slide (Figure 2C) shows that metallic silver can be deposited as nano-sized islands or particles onto the glass surface. Due to the noble metals' large extinction coefficients (sum of the absorption and the scattering coefficients),^{27, 28} optical absorption spectroscopy is employed to measure the optical properties (mainly surface plasmon resonance (SPR) peak(s)) of the metals. In this regard, zinc, silver and gold show a strong SPR peak at 375 nm, 420 nm and 520 nm, respectively (Figure 2D). Since surface plasmons afford for the enhanced emission in MEC (and MEF), for MEC-based applications, the wavelength of the SPR peak can be utilized to select an appropriate chemiluminescent/fluorescent species. In this regard, the absorption and scattering component of the metals extinction are two major factors influencing the selection of the chemiluminescent/fluorescent species.²² In reports on MEF to date, fluorescent species which radiate at red-shifted wavelengths with respect to the wavelength of the SPR peak of the metals are typically used. It is also important to note that the SPR peak strongly depends on the size and shape of the metal nanoparticles and the distance between the metal nanoparticles themselves.^{29, 30} One can also employ computational methods, such Mie calculations, discrete dipole approximation (DDA)³⁰ and finite difference time domain (FDTD)²² methods to calculate absorption/scattering efficiencies of metal nanoparticles and electric field distributions around metallic nanostructures.

Figure 3A shows a typical experimental geometry used in MEC studies. In this experimental geometry, a small amount ($\approx 70 \mu\text{L}$) of chemiluminescent reagent is placed in between two SIFs-deposited glass microscope slides, which are held together tightly with clamps placed on each end of the slides (sandwich geometry). The chemiluminescence emission spectra for blue (from the chemical reaction between hydrogen peroxide and 9,10-Diphenylanthracene) and green (9,10-Bis(phenylethynyl)anthracene) chemiluminescent species from between the silvered and unsilvered portions of the glass microscope slide are shown in Figures 3B and 3C. The emission from the silvered portion of the slide was spatially averaged to be about ≈ 4 and 10 times greater than the glass control side of the sample for blue and green chemiluminescent species, respectively. It is important to note that the chemiluminescent reagent was equally distributed in between the sandwiched glass microscope slides. Visual evidence for enhanced emission was provided by real-color photographs of the chemiluminescent emission from the glass microscope slide (Figure 3B-inset, 3C-inset). The enhanced chemiluminescence emission is clearly visible on the silvered portion, but is very weak from the glass portion of the slide. It should be noted that the *true* MEC enhancement factor here is much larger than 4–10-fold for the blue and green chemiluminescence species. Since most of the chemiluminescent species is in the bulk (the sample is 1 micron thick) and the MEC coupling region is thought to be ≈ 20 nm from the surface of the metal nanoparticles,^{15, 16} then only $\approx 2.5\%$ of the sample is within the MEC region; hence the true enhancement factor is approximately 40 times larger (i.e., up to 80-fold and 200-fold MEC for blue and green chemiluminescence species, respectively).

As described in the Introduction, chemiluminescence reactions require a catalyst (chemical or biological). In this regard, to investigate whether silver nanoparticles have any catalytic effect on the chemiluminescence emission, several key experiments were undertaken, where the chemiluminescence emission intensity from glass, SIFs and a continuous silver film were recorded as a function of time (chemiluminescence decay, Figure 4). Glass is considered to be a non-catalytic surface. Figure 4A shows the rates of loss of chemiluminescence on glass and SIFs was 0.019 vs 0.034 s^{-1} respectively. That is, the rate of depletion of chemiluminescence emission on SIFs was 1.7 times faster than on glass, which could be initially described by two explanations: 1) silver catalysis of the chemiluminescence reaction, 2) the high rate of transfer/coupling of the chemiluminescence to surface plasmons, rapidly reducing the excited state lifetime of the chemiluminescence species. To eliminate the silver nanoparticle-based catalysis

of the chemiluminescence reaction as an explanation for the enhanced chemiluminescence emission, the intensity decay rates on both SIFs and a continuous silver strip were also measured. Interestingly, the rate of loss of chemiluminescence was still found to be greater on the SIFs as compared to the continuous silver strip, Figure 4B. This suggests that silver nanoparticles have little or no catalytic effect on chemiluminescence reactions. Subsequently, these observations suggest that chemically induced electronic excited states (chemiluminescence emission) can readily induce/couple to surface plasmons, facilitating metal-enhanced chemiluminescence. Interestingly, MEC is a trough space phenomenon like MEF,^{18, 20} which suggests that coatings of inert materials can also be used to coat metals to alleviate potential catalytic effects.

In the past, our research laboratory has also reported MEF from metals other than silver, such as chromium,²³ zinc,²² copper,²⁴ and gold.²⁵ It is important to note that MEF phenomenon can only be observed with surface plasmon supporting metals. Based on the knowledge gained from these observations, we have also studied MEC from other surface plasmon supporting metals: chromium, copper, nickel and zinc. Figure 5 shows the enhancement factors for green chemiluminescence from these metals of various surface thicknesses. A typical enhancement factor of 2–3-fold is observed from many metal surfaces, which implies that chemically excited states can couple to these plasmon resonant metal particles as a means for enhancement. Interestingly, when the depleted chemiluminescent solutions are optically excited at 473 nm using a continuous wave source, for several particle sizes and metals, a greater enhancement factor (EF) is observed. Given that the enhancement factor is the ratio of the emission from metal divided by the emission from the glass control sample, then these increased enhancement factors are due to an increased electric field component, which is not present (or indeed vanishingly weak) when just chemiluminescent solutions are used. Note that for MEF where an optical excitation source is used

$$\text{MEF EF} = \text{Plasmon} - \text{coupled component} + \text{Electric field component} \quad (1)$$

or more simply,

$$\text{MEF EF} = \text{Enhanced emission} + \text{enhanced absorption} \quad (2)$$

For the standard chemiluminescence, only the plasmon-facilitated emission component is present.

It is also interesting to comment on the enhancement factor versus particle size data in Figure 5. In MEF, as the metallic surfaces become or approach being continuous, a reduced enhancement factor is typically observed,²¹ similar to what is observed in Figure 5.

3. Advanced Chemiluminescence Concepts: II. Microwave-Triggered Metal-Enhanced Chemiluminescence

Since early reports on the microwave-assisted synthesis of materials³¹ there has been a resurgence in the use of microwave processing of a variety of materials, such as the synthesis of polypeptides,³¹ semiconductors,³² organic compounds,³³ and inorganic compounds.³⁴ In all of these applications, microwave heating was shown to significantly accelerate chemical reactions, reducing the reaction times from hours to minutes.³⁵ Based on this knowledge, we questioned whether microwave heating can also accelerate chemical reactions resulting in enhanced chemiluminescence emission. In a recent paper,³⁶ our research laboratory has

demonstrated the proof-of-principle of a new technique called Microwave-Triggered Metal-Enhanced Chemiluminescence (MT-MEC), which significantly enhances the chemiluminescence emission and shortens the detection times by low-power microwave heating in the presence of SIFs.

Figure 6A summarizes the experimental details of the MT-MEC technique: a reagent containing the chemiluminescent species is placed on SIFs and is exposed to low-power microwave heating for a desired period of time. Chemiluminescence emission was quantitatively measured in terms of the total photon counts. To assess the benefits of microwave heating and also the presence of SIFs (i.e., MEC), several control experiments were also undertaken: 1) no microwave heating and 2) on glass (no SIFs). In addition, to demonstrate the “on-demand” nature of the MT-MEC technique, time-dependent chemiluminescent emission of a blue chemiluminescence reagent on SIFs (Figure 6B) and glass surfaces (Figure 6C), with multiple microwave exposures and without any microwave exposures (Figure 6B-inset and 6C-inset) was recorded and compared for 2000 seconds.

The exposure of the blue chemiluminescence reagent to microwaves (multiple exposures) results in an increase in the chemiluminescence emission, which is observed as “triggered spikes” consistent with the rising edge of the microwave pulses in the graph (Figure 6B and 6C). The largest increase (120 A.U. to 3300 A.U. on average) in chemiluminescence intensity was observed during the first five microwave exposures and diminished upon further exposures, as chemiluminescent reagent is depleted. In all the experiments performed with low power microwaves, using both SIFs and glass, there was no evidence of surface drying. This is attributed to the previously made observations that the temperature increase of the aqueous reagent on the surfaces due to microwave heating is only $\sim 8^{\circ}\text{C}$ (to $\approx 28^{\circ}\text{C}$) for a $30\ \mu\text{l}$ of aqueous sample. The initial intensities at time = 0 seconds for both graphs are $\approx 120\ \text{A.U.}$ The “triggered spikes” in the intensity, indicate the individual microwave exposure (10 seconds, 20% power). Figure 6B-inset and 6C-inset show the time-dependent emission (No microwave exposure) and the real-color photographs of the blue chemiluminescent reagent (before and after Mw exposure) on SIFs and glass surfaces.

The total number of photons detected from the blue chemiluminescent reagent on the SIFs and glass surfaces after microwave exposures in 2000 seconds is 351×10^3 and 281×10^3 counts, respectively (Figure 6B and 6C). The total photon counts are significantly higher than those obtained without microwave exposures, 143.5×10^3 and 114.5×10^3 counts for SIFs and glass, respectively (Figure 6B-inset and 6C-inset). This corresponds to a 2.45-fold increase in photon flux on both SIFs and glass surfaces. It is important to note that a 2.45-fold increase in photon flux represents the average increase in the overall photon flux from the ensemble of chemiluminescent species for 2000 seconds. One can complete the chemiluminescent reaction with a single microwave exposure for 10 seconds that will yield a similar final photon flux for the chemiluminescent reactions without microwave heating (i.e. 10 sec vs. 2000 sec). Considering the fact that the chemiluminescent reactions currently in use today are usually completed within 5 hours, the MT-MEC technique³⁶ provides researchers with an increased detectability (MEC and microwave accelerated) and a significant reduction in chemiluminescent detection time, to as low as 10 seconds.

As described above, MT-MEC technique affords for the acceleration of the chemical reactions and the collection of the blue chemiluminescence emission “on-demand” with significantly increased photon counts as quick as only a few seconds. Our research laboratory has also investigated the application of the MT-MEC technique to chemical reactions yielding colors other than blue to green and also red. In this regard, a solution of blue, green and red chemiluminescence reagents were placed on SIFs and a blank glass microscope slide and were subsequently exposed to 10 seconds of microwave heating (Figure 7A). The

chemiluminescence emission spectra and the real-color photographs of the chemiluminescence emission for all three reagents, before and after the microwave heating, were recorded (Figure 7B and 7C). The chemiluminescence intensity for all three reagents on SIFs and glass surfaces is enhanced significantly after the microwave heating, as also evidenced by the real-color photographs. After 10 seconds of microwave heating, the chemiluminescence intensity due to microwave heating was increased, ranging from 22 times (blue, on glass) to 85 times (green, on SIFs). The comparison of chemiluminescence intensity on SIFs after 10 seconds of microwave heating with the chemiluminescence intensity on glass before the microwave exposure (i.e., MT-MEC) shows that the microwave heating of the chemiluminescent species in the presence of silver increases the chemiluminescence intensity 54 times (blue) to 125 times (green) for 10 seconds of microwave heating.

Our research laboratory has also demonstrated the application of MT-MEC technique to chemiluminescence-based quantitative protein detection, where the chemical reactions resulting in chemiluminescence emission are catalyzed by a biological catalyst (i.e., an enzyme).³⁷ Figure 8A shows the schematic depiction of the model chemiluminescent assay for the detection of a protein (bovine serum albumin, BSA). In this assay, various amounts of biotinylated-BSA are incubated on SIFs and glass surfaces over a 30 minute period, where biotinylated-BSA is adsorbed (physical adsorption) to the surfaces. Subsequently, horse radish peroxidase (enzyme)-labeled avidin was added to the surface, localizing the enzyme catalyst in close proximity to the surfaces noting that MEC is a close-range but through space interaction. Then, other reagents (hydrogen peroxide and acridan) are added to initiate the chemiluminescence reaction. After the initiation of the chemiluminescence reactions, the assay was exposed to multiple microwave pulses and the assay was allowed to run for 500 seconds while chemiluminescence emission intensity (photon counts) was measured (Figure 8B). To quantitatively assess the amount of biotinylated-BSA detected, the total number of photons (500 seconds) was counted and was plotted against the concentration of biotinylated-BSA (Figure 8C). Figure 8C shows the sensitivity of the MT-MEC assay with respect to the integrated background counts on SIFs and glass substrates (straight dashed lines) and also that the microwave-triggered emission intensity can be used for quantitative protein detection.³⁷ From these observations, one can see that the implementation of low power microwaves increases the detectability in protein based assays, and could equally be applied to the detection of DNA and RNA's as well.

4. Advanced Chemiluminescence Concepts: III. Surface Plasmon Coupled Chemiluminescence

Our, and many other research group's continued efforts to understand the interactions of light with metallic surfaces which support surface plasmons has led to development of several techniques based on MEF and MEC. These techniques utilize metallic nanoparticles that significantly increase the fluorescence or chemiluminescence emission, which in turn increases the detectability in biological assays. In addition to metallic nanoparticles, thin films of the metals which support surface plasmons are also known to interact with light. Surface plasmon resonance (SPR) is example of light-metal thin film interactions.³⁸ In SPR, the change in angle of the reflected light from metal thin film provides quantitative information on the specific biological recognition events occurring in close proximity to the metal surface. After its introduction in the early 1990s, the SPR technique has become a dominant technique in the detection of a wide range of biological and chemical materials of importance. In the late 1990's, another technique, called Surface Plasmon Fluorescence Spectroscopy (SPFS), which combines fluorescence spectroscopy and the SPR technique was introduced.³⁹ In SPFS, fluorescently-labeled biomolecules are brought in close proximity to the surface of the metal thin films via biorecognition events between metal surface bound biomolecules and the

fluorescently-labeled biomolecules, as part of the bioassays constructed on the metal surface. The fluorescence emission is then detected from the sample side or through the prism and is used to quantify the biomolecule of interest. In this regard, attomolar sensitivity in immunoassays based on SPFS has been reported.⁴⁰ SPSF has also been used for DNA hybridization^{41–43} and protein detection.⁴⁴

Our research laboratory recently reported a companion technique called surface plasmon coupled chemiluminescence (SPCC), which is based on the interactions of chemiluminescence emission with metal thin films.^{45, 46} In SPCC, chemically induced electronic excited states induce surface plasmon modes in metal thin films and the coupled emission is subsequently emitted from the back of the metal thin films both in a highly directional and polarized fashion.⁴⁶ In close proximity to the metal surface (< 200 nm) SPCC emission is larger than that of free-space emission due to efficient coupling of emission to surface plasmons. Interestingly, most biological assays that are constructed on planar surfaces require the placement of enzymes close to surfaces through biological recognition events, the resulting chemiluminescence emission typically occurs within a few hundred nanometers away from the surface in any case which supports the SPCC approach.

The proof-of-principle of the SPCC technique was recently demonstrated with a blue chemiluminescent reagent and silver thin films.⁴⁶ Figure 9A depicts the experimental geometry used in SPCC. SPCC measurements are performed in the following manner: a silver thin film (47 nm thick) coated glass support is placed on a prism (hemispherical or right-angle) and the chemiluminescent reagent is placed on the silver thin film. It is important to note that hemispherical prism is employed for the collection of emission at all angles and right-angle prism is typically employed for the collection of emission at a fixed angle. Chemiluminescence emission from the sample side (free-space emission) and from the back of the prism (SPCC) was collected with a detector (Figure 9B). While an isotropic free-space emission was observed from the sample side, the SPCC emission was highly directional and *p*-polarized. The chemiluminescence emission spectra measured from both free-space and SPCC was similar, implying that the near-field emission is unaltered. This important observation demonstrates that typically isotropic chemiluminescent emission can be made highly directional by employing thin metal film substrates. In many chemiluminescent-based assays, the majority of the emission is lost due to inefficiencies of the collection optics. SPCC allows for a much greater collection of chemiluminescent-based photons, potentially increasing detection/analyte sensitivity, all in a thin-film format, not unlike traditional chemiluminescence based assay approach.

5. Conclusions

Chemiluminescence is a useful analytical technique for the detection of biological and chemical species. The ever-growing demand for better, faster and more sensitive detection of these species has led to several new chemiluminescence concepts. These new concepts are based on our developing understanding of the interactions of light with metallic surfaces. Metal-Enhanced Chemiluminescence, where chemiluminescence emission can be significantly increased with the use of metallic nanoparticles, affords for more sensitive chemiluminescence-based bioassays to be developed. The speed of the chemiluminescence-based bioassays can also be reduced from hours to seconds with the incorporation of microwave heating in Microwave-Triggered Metal-Enhanced Chemiluminescence. Chemiluminescence emission can also be made directional and polarized with Surface Plasmon Coupled Chemiluminescence for even more sensitive chemiluminescence-based bioassays. The practical implementations of these new concepts will undoubtedly improve the already proven chemiluminescence technology for the 21st century.

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Figure 1.

Chemiluminescence basics. (A) Chemiluminescence is a result of chemical reactions between two or more compounds: for example; the reaction of luminol and hydrogen peroxide yields a blue emission at 450 nm. (B) A graph depicting the time-dependent nature of traditional chemiluminescence emission. (C) Real-color photographs of red (as a result of oxidation of 5,12-Bis(phenylethynyl)naphthacene), green (9,10-Bis(phenylethynyl)anthracene) and blue (9,10-Diphenylanthracene) chemiluminescence.

Figure 2. Schematic depiction of (A) Metal-Enhanced Chemiluminescence (MEC) and (B) the Metal-Enhanced Fluorescence (MEF) phenomenon. (C) Atomic Force Microscope image of silver island films (SIFs) on a glass slide. (D) Normalized extinction of zinc, gold and silver nanostructured particles on a glass slide. F-Fluorophore, FL- Fluorescence, CL- Chemiluminescence.

Figure 3.

(A) Schematic depiction of the experimental geometry for Metal-Enhanced Chemiluminescence (MEC) studies. Chemiluminescence spectra of (B) blue and (C) green chemiluminescence dyes on glass and SIFs. The insets show the photographs of the actual glass microscope slide with SIFs and the chemiluminescence dyes placed in between two silvered glass microscope slides. The enhanced chemiluminescence emission can clearly be seen from silvered portion of the slide. SIFs- Silver Island Films.

Figure 4.

The decay of emission intensity of blue chemiluminescence dye placed on (A) SIFs and glass and (B) a continuous strip of silver film, versus time. The decay rates were calculated by fitting the data first order exponential curve.

Figure 5. Enhancement factor versus thickness of the metal film for (A) chromium (Cr), (B) copper (Cu), (C) nickel (Ni), (D) zinc (Zn) for both a green chemiluminescence solution (Green CL) and, “445 nm laser” refers to the the same solution after reaction completion but optically excited at $\lambda_{\text{ex}} = 445 \text{ nm}$.

Figure 6.

(A) Schematic depiction of microwave-triggered chemiluminescence and the microwave-triggered metal-enhanced chemiluminescence phenomena. Total photon counts are used to compare the efficiency of chemiluminescence emission on SIFs and glass with and without microwave heating. Time-dependent microwave-triggered chemiluminescence emission (intensity: A.U.-arbitrary units) for a blue chemiluminescent dye on (B) SIFs and (C) glass surfaces before, during and after low power microwave (Mw) heating.

Figure 7.

(A) A schematic depiction of multi-color microwave-triggered metal enhanced chemiluminescence. Chemiluminescence emission spectra (intensity: A.U.-arbitrary units) of blue, green and red chemiluminescent reagents on (B) SiFs and (C) glass surfaces before and after 10 seconds microwave (Mw) exposure. The inserts show the spectra (before the Mw exposure) and the real-color photographs of the chemiluminescent reagents (before and after the Mw exposure).

Figure 8.

Microwave-triggered metal enhanced chemiluminescence based detection of proteins on SIFs and glass microscope slides. (A) Horse Radish Peroxidase (HRP) chemiluminescence assay on both glass and SIFs. (B) 3D plots of the chemiluminescence emission as a function of time from SIFs (Top) and glass (Bottom) with multiple low power microwave pulses. (C) Integrated photon flux of chemiluminescence emission for different concentrations of biotinylated-BSA from both SIFs and glass. Straight lines depict the background counts from the control assay (no biotinylated-BSA).

Figure 9. (A) Surface Plasmon Coupled Chemiluminescence (SPCC) from 47 nm thick silver films. (B) Free space emission and SPCC, (C) Emission spectra of both the free space emission and SPCC.