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Research paper

# Microwave-accelerated surface plasmon-coupled directional luminescence 2: A platform technology for ultra fast and sensitive target DNA detection in whole blood

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**Abstract**

The application of Microwave-Accelerated Surface Plasmon-Coupled Luminescence (MA-SPCL) to fast and sensitive DNA hybridization assays in buffer and whole blood is presented. In this regard, a model DNA hybridization assay whereby a fluorophore-labeled target ssDNA specific to human immunodeficiency, *Hepatitis C* (Hep C), is probed by an anchor probe immobilized on thin gold films, is driven to completion within 1 min with microwave heating, as compared to an identical assay completed in ≈4 h at room temperature. Finite-Difference Time-Domain calculations show that gold disks are preferentially heated around the edges creating a temperature gradient along the disks, which in turn results in the larger influx of complementary DNA towards anchor probe-modified surface. Thermal images of the assay platform during microwave heating also provide additional information on the microwave heating pattern in the microwave cavity. Finally, the effects of low power microwave heating on the ability of DNA to re-hybridize with the complimentary target on the surface gold films, which allows the multiple re-use of the gold films, is demonstrated. The MA-SPCL technique offers an alternative approach to current DNA based detection technologies, especially when speed and sensitivity are required, such as in the identification of DNA or even RNA-based diseases using whole blood samples that affect human health.


*Keywords:* Microwave-accelerated metal-enhanced fluorescence; Metal-enhanced fluorescence; Surface plasmon fluorescence spectroscopy; Surface plasmon-coupled emission; Low power microwaves; Ultra fast assay kinetics; Surface assays; Chemiluminescence; Surface-enhanced fluorescence; Radiative decay engineering

## 1. Introduction

DNA hybridization assays are routinely used in a variety of biotechnology and diagnostics applications (Morrison, 2003), such as gene chips (Brown and Botstein, 1999) during PCR (Komurian-Pradel et al., 2001; Walker, 2002; Difilippantonio and Ried, 2003) and fluorescence-based in situ hybridization (Difilippantonio and Ried, 2003). It is most desirable to have these

*Abbreviations:* FDTD, Finite-Difference Time-Domain; Hep C, *Hepatitis C*; MA-SPCL, Microwave-Accelerated Surface Plasmon-Coupled Directional Luminescence; SPCL, Surface Plasmon-Coupled Directional Luminescence; SPR, Surface Plasmon Resonance; SPFS, Surface Plasmon Fluorescence Spectroscopy.

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applications employ DNA hybridization assays that are sensitive, specific and in some cases, rapid. The sensitivity of the fluorescence-based DNA hybridization assays is affected by multiple factors, which include the low signal-to-noise ratio at low analyte concentrations and the lack of photostability of the fluorescent probe used (Aslan et al., 2005). Moreover, DNA hybridization assays are often kinetically slow and require long incubation times (Kessler, 2007; Stelzl et al., 2007). Therefore, there is an unequivocal need for the development of more sensitive and faster detection methods for more efficient DNA hybridization assays.

Surface Plasmon Fluorescence Spectroscopy (SPFS), a technique introduced by Knoll's research group in 2000 (Liebermann and Knoll, 2000; Liebermann et al., 2000), combines fluorescence spectroscopy and surface plasmon resonance (SPR) technologies and offers a more sensitive alternative to conventional DNA detection assays (Ekgasit et al., 2004; Tawa et al., 2005). In this regard, while SPR allows the observation of reactions taking place on the gold surface via the change in refractive index, one can detect the directional fluorescence emission coupled to surface plasmons (Liebermann and Knoll, 2000). More specifically, during a biorecognition event at the metal/solution interface, the fluorophore is exposed to the strong optical fields giving rise to significant enhancement in fluorescence emission that is highly polarized and directional. The emitted fluorescence emission is monitored and correlated to the concentration of the analyte (Liebermann et al., 2000). The use of fluorescence detection schemes in combination with surface plasmons has been shown to increase the sensitivity for bioanalyte monitoring considerably (Stengel and Knoll, 2005).

Although SPFS provides one with the sensitivity required for DNA hybridization assays, one does not have control over the assay run time, which is governed by the thermodynamics of the hybridization process. In this regard, our laboratory recently described a new platform technology microwave-accelerated surface plasmon-coupled directional luminescence (MA-SPCL). MA-SPCL combines the use of microwave heating and surface plasmon-coupled luminescence to develop fast and sensitive bioassays in human serum and whole blood (Aslan et al., 2007). This was demonstrated with a model protein bioassay based on biotin–streptavidin interactions constructed on a 50 nm thick gold film. In that paper, streptavidin-conjugated quantum dots were used as the luminescent reporter molecules, where the surface plasmon-coupled *directional luminescence intensity* was measured and correlated to the amount of streptavidin detected. The MA-SPCL assays allowed the detection of streptavidin in buffer, human serum and whole blood within 1 min, with the use of low power microwave heating, where the same assay took >30 min to reach >95% completion at room temperature.

In this paper, we have investigated the applicability of the MA-SPCL platform technology to the fast and sensitive detection of *DNA* in buffer and whole blood samples. We have used *Hepatitis C* (Hep C) specific DNA in the *model* DNA hybridization assay for MA-SPCL based detection of target DNA. In this regard, the hybridization of TAMRA-labeled target Hep C ssDNA with an anchor probe on the surface of a gold film that is specifically designed for microwave heating was completed in 1 min in a microwave cavity, while the identical assay took ≈4 h at room temperature. Highly polarized and directional fluorescence emission was detected at surface plasmon coupling angle from the back of the gold film. We have shown that samples containing as low as 10 nM Hep C ssDNA in buffer and whole blood can be detected within 1 min with the MA-SPCL platform technology. As a result, this technology delivers a versatile DNA hybridization assay that is rapid, sensitive and can be performed in complex media formulations. We have also shown that the gold films can be reused for sequential hybridization assays by melting and re-hybridization studies. We also employed Finite-Difference Time-Domain (FDTD) calculations and a thermal imaging camera to better understand the microwave heating process that results in the ultra fast DNA hybridization kinetics. To the best of our knowledge, this is the fastest surface-plasmon-coupled luminescence-based detection of target DNA reported to date and is applicable to *any* target DNA detection assays currently in use.

## 2. Materials and methods

### 2.1. Materials

Premium quality plain glass microscope slides (75×25 mm) were obtained from Sigma-Aldrich. Oligonucleotides used in this work were designed from a previously published sequence for Hep C (Stuyver et al., 1996) and were obtained from MWG-Biotech AG (High Point, NC). Tris (10 mM)/EDTA (1 mM) 1×, pH 7.6 solution was purchased from Fisher Scientific. All materials were used as received.

### 2.2. Preparation of the metal films for surface plasmon-coupled luminescence (SPCL) spectroscopy

This procedure was published previously (Aslan et al., 2007). In short, a glass slide is coated with a 50 nm

thick gold film by vapor deposition and cut into small pieces ≈12 mm square. Then, the gold film is removed by a "lift-off technique" except for a 5 mm circular disk (Fig. 1A). A "black body" is then applied to the bare glass around the circular gold disk (Aslan et al., 2007).

### 2.3. Microwave-accelerated SPCL DNA hybridization assay for Hepatitis C

MA-SPCL DNA hybridization assays for *Hepatitis C* (Fig. 1A) were performed in two steps: i) attachment of the anchor probe to the gold films and, ii) hybridization of fluorophore-labeled target oligo with anchor probe on the surface of the gold films with and without microwave heating. All oligonucleotides were heated at 80 °C for 2 min and kept in ice until further use. The anchor probe is attached to gold surface via overnight incubation of 30 µl 10 µ*M* anchor probe solution in the "black body" micro cuvettes at 4 °C, followed by rinsing with deionized water to remove the unbound material. For the DNA hybridization assays, then a 30 µl of varying concentrations of TAMRA-labeled target ssDNA (in Tris/EDTA pH 7.6 buffer) was subsequently added into the anchor probe-coated gold films for 4 h for the control experiments at room temperature, and 1 min in the microwave cavity (0.02 $m^3$, GE Compact Microwave Model: JES735BF, max power 700 W). The power setting was set to 3. In all the experiments performed with low power microwaves, using metal film-coated micro cuvettes modified

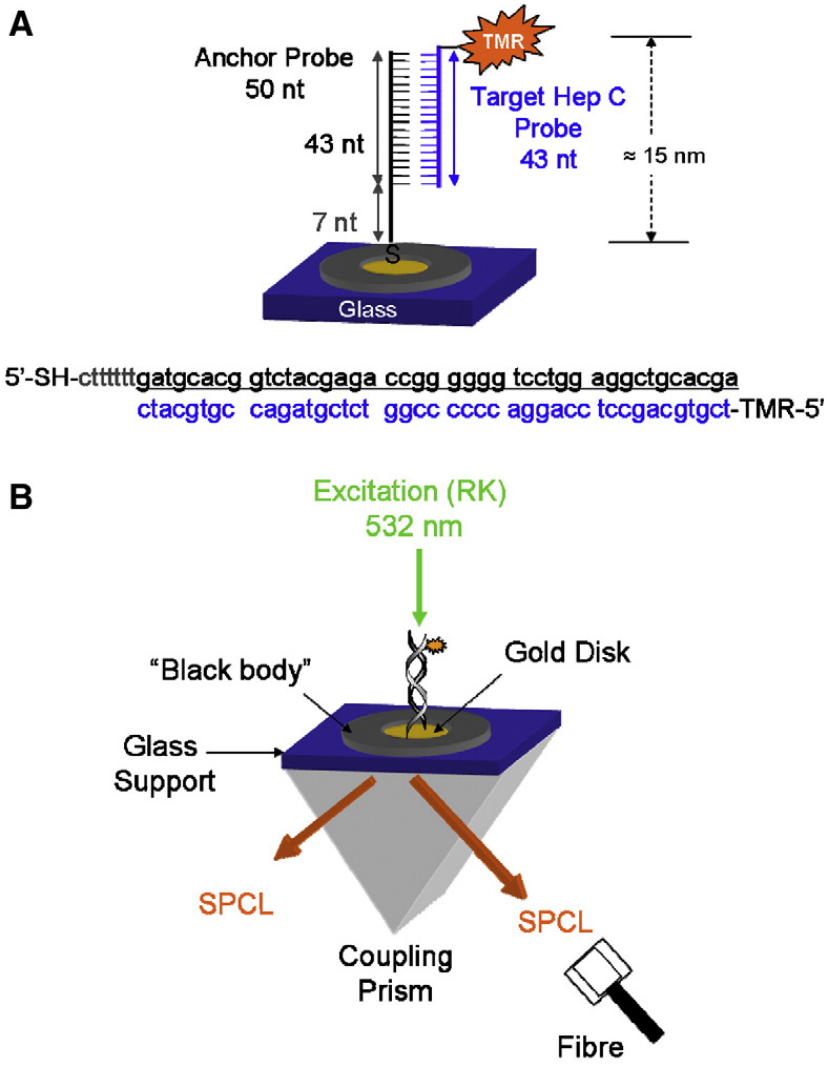


Fig. 1. Experimental setup. (A) Experimental design depicting the organization of the DNA oligomers on gold disks used for the detection of the model *Hepatitis C* (Hep C) assay. The lower panel shows the structures of the DNA oligomers used (conserved sequence for Hep C is shown). (B) Optical setup for microwave-accelerated surface plasmon-coupled luminescence (MA-SPCL) bioassays. The sample is excited in the reverse Kretschmann (RK) configuration. The assay is undertaken on the gold-coated glass slide, which is attached to the glass prism with index matching fluid. The two arrows on the left side show the directional coupled luminescence emission. Figures are not drawn to scale.

with the "black body", there was no evidence of surface drying. Detailed information about the "black body" is given elsewhere (Aslan and Geddes, 2005).

DNA hybridization assays in whole blood were performed similar to the procedure explained above, 15 µl of varying concentrations of TAMRA-labeled target ssDNA were mixed with 15 µl of whole blood, and this mixture added onto the anchor probe-coated gold films for 1 min within the microwave cavity. The mixture was then washed several times with deionized buffer.

### 2.4. Melting of DNA and re-hybridization with microwaves

The melting of the DNA (melting temperature ds-DNA: 76 °C) was accomplished by incubating the DNA in 40 ml of Tris/EDTA buffer at 80 °C for 20 min with the buffer being replaced every 60 s, effectively washing the target Hep C ssDNA at high temperature. The re-hybridization of 1000 nM TAMRA-labeled ssDNA with the anchor probe-coated on the gold films was achieved using microwave heating for 1 min, as described above.

SPCL measurements were made as follows: the DNA-coated gold films were attached to a right-angle prism made of BK7 glass with index matching fluid (Fig. 1B). This combined sample was positioned on a precise rotary stage ($x$–$z$) that allows excitation and observation at any desired angle relative to the vertical axis ($z$-axis) along the prism. The sample was excited using the Reverse Kretschmann configuration from the air or sample side, which has a refractive index lower than the prism. The excitation was from the second harmonic (532 nm) of the diode-pumped Nd:YVO4 laser at an angle of 90°. Observation of the emission was performed with a 3 mm diameter fiber bundle, covered with a 200 µm vertical slit, positioned about 15 cm from the sample. This corresponds to an acceptance angle below 0.1°. The output of the fiber was connected to an Ocean Optics HD2000 spectrofluorometer to measure the florescence emission spectra through a 532 nm super notch filter (Semrock).

### 2.5. FDTD simulations and theory

Three-dimensional gold disks, 5 mm in diameter and 5 µm thick created using CAD in the FDTD simulation software (Lumerical Solutions, Vancouver, B.C.). There is negligible transmission of radiation through the thin metallic films due to the negligible penetration of the electromagnetic fields into the perfectly conducting metal layer (Suckling et al., 2007). While the actual thickness of the gold disk is approximately 50 nm, the relative field distributions for decreased aspect ratios will adequately reflect the expected field distributions for the thin film geometries. Subsequently, to avoid excessive computational times, we decreased the aspect ratio of the disc geometries such that the diameter of the gold discs were 5 mm and 5 µm thick. Convergence testing was performed by varying the mesh size in the $x$ and $y$ dimension from 100 to 60 µm at 10 µm intervals and from 5 µm to −5 µm at 0.1 µm intervals (data not shown).

Optical properties of perfectly conducting metal structures have been described previously (Previte and Geddes, 2007). Briefly, the total complex permittivity of a perfect conducting metal, such as gold, in the presence of a microwave field is given by

$$\tilde{\varepsilon}(f) = \varepsilon_{\mathrm{REAL}} + i\varepsilon_{\mathrm{IMAG}}\frac{f_{\mathrm{SIM}}}{f} + i\frac{\sigma}{2\pi \cdot f\varepsilon_{\mathrm{o}}} \quad (1)$$

where $\varepsilon_{\mathrm{REAL}}$ is the real part of the permittivity for the dielectric medium, $\varepsilon_{\mathrm{IMAG}}$ is the imaginary of the dielectric medium, $f_{\mathrm{SIM}}$ is the center frequency of all the sources in the simulation, $f$ is the frequency of the simulation, $\varepsilon_{\mathrm{o}}$ is the permittivity of free space, and $\sigma$ is the conductivity of the metal (Lumerical Solutions, 2006) The conductivity value of the metal is approximated to be close to the d.c. value for gold, $4.1 \times 10^7$ S m$^{-1}$ (Suckling et al., 2004), the background dielectric media is presumed to be air, and the frequency, $f$, is set to 2.45 GHz.

As previously described, we modeled this microwave system as a total field scattered electric field that propagates along the $x$ dimension to best approximate the electromagnetic field in the microwave cavity during operation and assumed only $TE_{10}$ modes could propagate in the microwave cavity (Iwabuchi et al., 1996; Previte and Geddes, 2007). The simulation time is set to 10 ns to insure that the light travels down the surface and back. The absorbing boundary conditions are of the perfectly matched layer type and are used to truncate the FDTD domain in the $x$ and $y$ dimensions (Lumerical Solutions, 2006).

### 2.6. Thermal imaging setup

Thermal imaging is accomplished using the following procedure: A 2.54 cm diameter opening is cut through the bottom of the microwave cavity and all exposed metal surfaces are coated with white enamel reflective paint. Sapphire plates (Swiss Jewel), 2.54 cm in diameter and 1 mm thick, are placed above the cavity opening as sandwich geometries to thermally image the temperature distributions of the glass and Au disk

samples, whereby 50 µl of water is dropped on a blank sapphire plate and the substrates placed on the sapphire plate to create a sandwich. For the glass substrates with the 5 mm Au disk 50 nm thick, the samples are inverted such that the Au disk is in contact with the water. Infrared emission from the sample geometry in the microwave cavity is imaged by reflecting the radiation from a gold mirror onto a thermal imaging camera (Silver 420 M; Electrophysics Corp, Fairfield, NJ) that is equipped with a close-up lens that provides a resolution of approximately 300 µm.

### 2.7. Thermal imaging experimental conditions

Images were collected at 50 Hz for 20 s. Samples were exposed to 15 s microwave pulses that were applied immediately following the commencement of thermal image collection. Timing graphs are reflective of the mean intensity temperature over the entire area of the respective outlined regions. Since initial sample temperatures were slightly variable, the temperature timing graphs are normalized with respect to the initial temperature of the respective samples. These values are then appropriately scaled to reflect the relative change in temperature of the surfaces. Example thermal images are *true temperature images* that correlate with discrete time points of the overall image collection time.

## 3. Results and discussion

It was previously shown that coupled luminescence is expected to be predominantly *p*-polarized (Lakowicz,

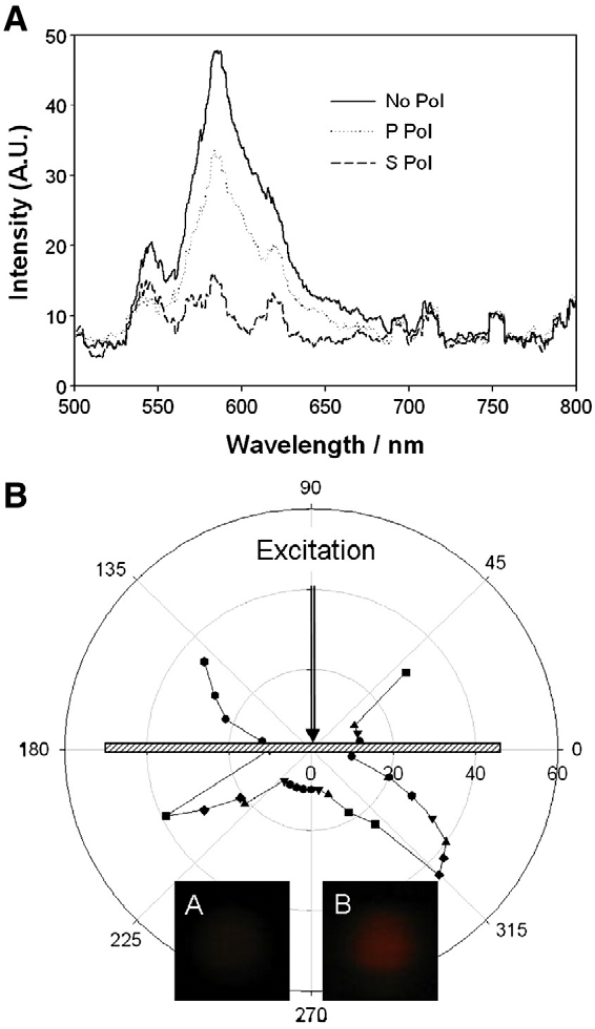


Fig. 2. Surface plasmon-coupled luminescence measurements. (A) Polarization dependent emission from 100 nM TAMRA-labeled target DNA used in the MA-SPCL assay. The emission measured through the gold disk is predominantly *p*-polarized. The assay was microwave-accelerated and the hybridization was kinetically completed <1 min. (B) Angular distribution of luminescence for 100 nM of TAMRA-labeled target DNA used in the MA-SPCL assay. Photographs (A) and (B) are showing TAMRA emission at 310 and 315° obtained through the same emission filter used in (B). No Pol: No polarization.

2004). In this regard, to demonstrate that the luminescence that is measured in our system is in fact plasmon-coupled, the polarization-dependent emission from 100 nM TAMRA-labeled target *Hepatitis C* DNA (MA-SPCL assay) is measured (Fig. 2A). As expected, the emission measured through the gold film was

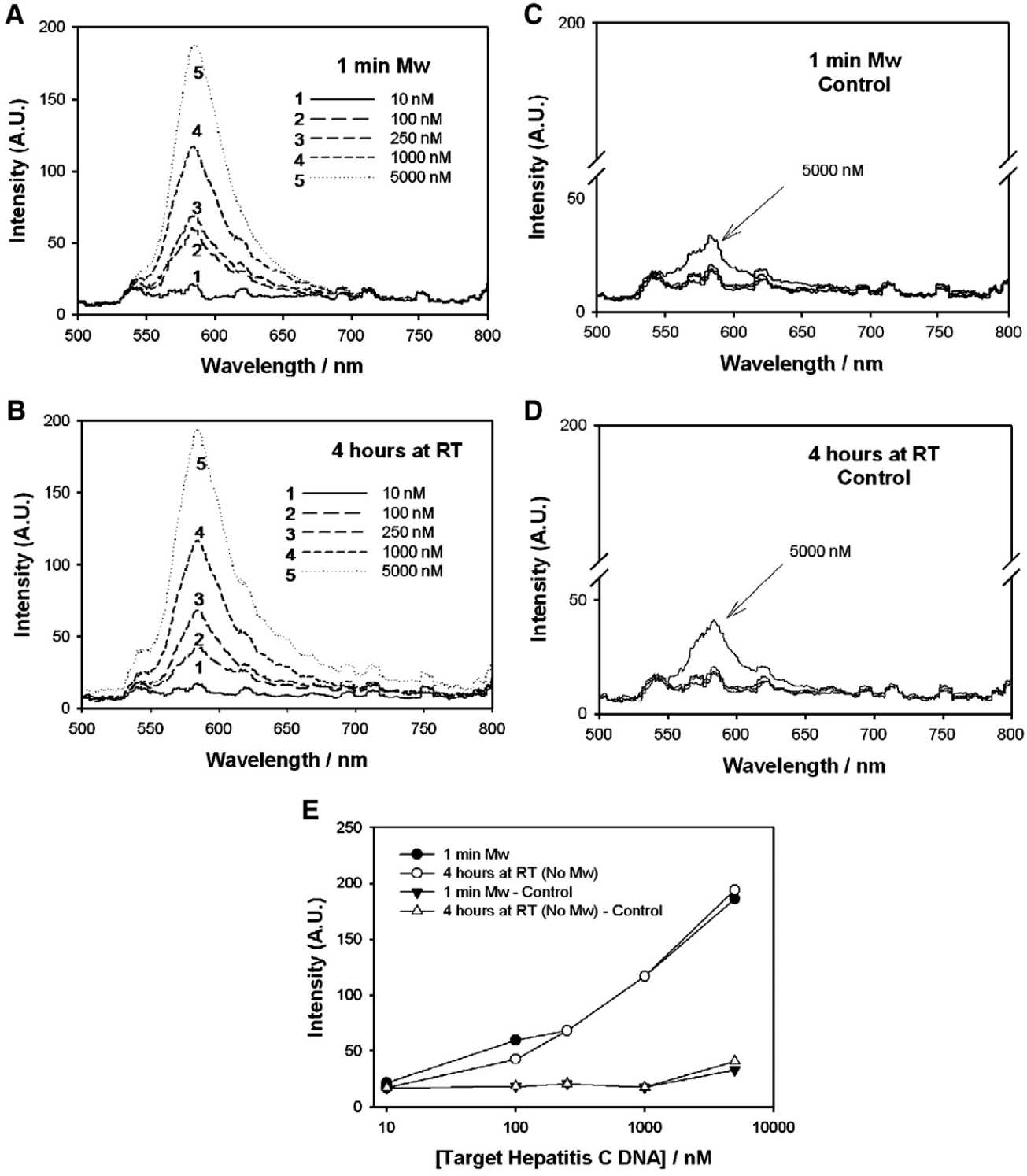


Fig. 3. MA-SPCL experiments. The emission spectra of varying concentrations of TAMRA-labeled target DNA used in the MA-SPCL assay (A) and the same assay measured at room temperature, RT, (B) all measured at 315°. The emission spectra of varying concentrations of TAMRA-labeled target DNA used in the MA-SPCL control assay (C) and room temperature control assay (D) measured at 315°. The anchor probe was omitted from the surface in these control assays. (E) Calibration curves for the MA-SPCL and room temperature Hep C assay with respective control experiments/samples, from Fig. 3A and B and Fig. 3C and D. Mw — Microwave heating.

predominantly *p*-polarized. We note that the *s*-polarized emission spectrum shown in Fig. 2A is identical to the background (no fluorophore on the surface), which is consistent throughout the text and accounts for the larger intensity observed for the MA-SPCL assay when no polarizer is used. Thus, no polarizers were used for the remainder of the data presented here.

We note that the gold films employed in the MA-SPCL assays are designed for microwave heating using a "lift-off" technique previously described by the authors (Aslan et al., 2007). These gold films are ≈5 mm in diameter, which is significantly smaller than the wavelength of the microwaves at 2.45 GHz (12.3 cm). Consequently, we do not observe surface sparking and arcing due to the surface charge buildup on the gold films (Whittaker and Mingos, 1993).

Fig. 2B shows the angular distribution of luminescence for 100 nM of TAMRA-labeled target DNA used in the MA-SPCL assay. The surface plasmon-coupled luminescence (observed through a prism between the angles of 180 and 360°, is directional at two angles, 205 and 315°. Fig. 2B also shows the free-space luminescence measured between 0 and 180°. We note that the free-space luminescence between 40 and 140° were not measured due to the obstruction of these angles by the experimental fiber holder. Photographs (A) and (B) show TAMRA emission at 310 and 315° obtained through the same emission filter used in Fig. 2B. The photographs provide visual evidence for the directionality of plasmon-coupled luminescence.

Fig. 3 shows the emission spectra of varying concentrations, 10–5000 nM, of TAMRA-labeled target DNA used in the MA-SPCL assay (A) and the same assay measured at room temperature (B) all measured at 315° from the back of the film. The assay yields similar final luminescence intensities for all the concentrations, after 1 min microwave heating as compared to 4 h room temperature incubation. Remarkably, the same extent of hybridization is evident after just 1 min of microwave heating. This demonstrates the utility of the MA-SPCL technique and that ultra fast directional luminescence DNA hybridization assays in quantitative manner can be realized by our technique.

Control experiments, where the anchor probe is omitted from the surface were also undertaken to determine the extent of non-specific binding of target DNA to the surface. In this regard, the emission spectra of varying concentrations of TAMRA-labeled target DNA used in the MA-SPCL control assay (A) and room temperature control assay (B) measured at 315° are shown in Fig. 3C and D. Fig. 3C and D shows that control experiments did not yield any luminescence signal (for 10–1000 nM) since the target DNA could not bind to the surface in the absence of the anchor probe. However, a slight increase in the non-specific binding for 5000 nM target DNA was observed. Careful examination of Fig. 3C and D reveals that the extent of non-specific binding in MA-SPCL is reduced compared to the identical control assay run at room temperature, a reproducible finding. This suggests that the microwave heating has an influence on the extent of non-specific binding in the control experiments. We note that a reduction in non-specific binding in the control experiments directly translates into better sensitivity in the DNA hybridization assays.

The calibration curve (fluorescence emission intensity at 585 nm measured at 315° in the MA-SPCL setup vs. concentration of the target DNA) is obtained from

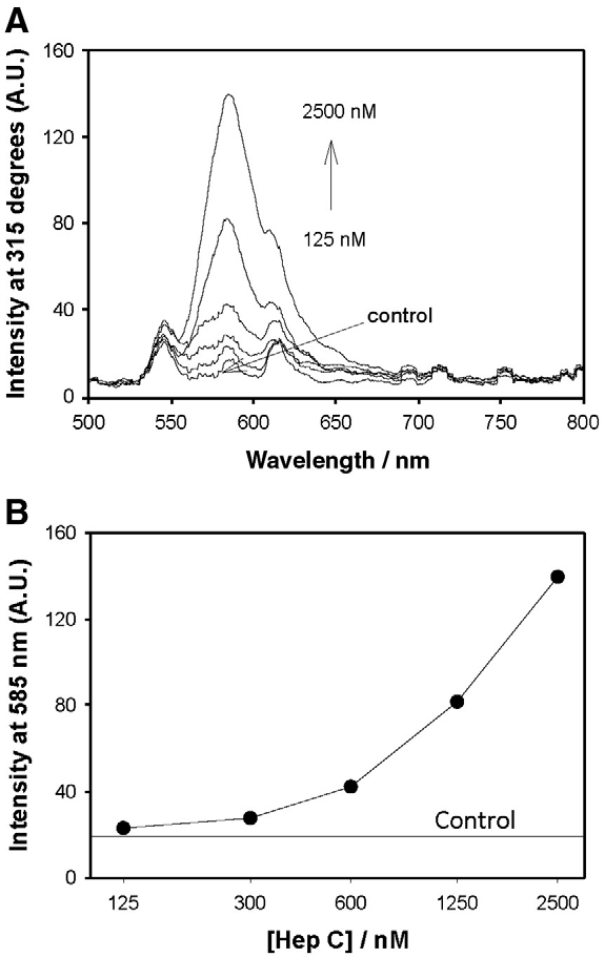


Fig. 4. Whole blood MA-SCPL experiments. The emission spectra (measured at 315°) for varying concentrations of TAMRA-labeled target DNA used in the MA-SPCL assay run in whole blood (A). The calibration curve, semi-logarithmic plot of intensity at 585 nm vs. concentration of the target Hep C DNA, is obtained from the top figure (B). Control experiment: 2500 nM.

the Fig. 3A, B, C and D and is shown in Fig. 3E. Fig. 3E shows that, the intensity measured at 585 nm increases with the increasing concentration of target DNA while the intensity for control experiments remained ≈ constant.

The applicability of the MA-SPCL technique to DNA hybridization assays in whole blood was also investigated. In this regard, target *Hepatitis C* specific ssDNA is mixed with whole blood (50% v/v) and this mixture was incubated on the anchor probe-coated gold films for 1 min in the microwave cavity. Fig. 4 shows the emission spectra (measured at 315°) for varying concentrations of TAMRA-labeled target ssDNA used in the MA-SPCL assay run in whole blood (A) and the calibration curve, a plot of intensity at 585 nm vs. concentration of the target *Hepatitis C* DNA that was obtained from Fig. 4A (B). A control experiment, where 2500 nm target *Hepatitis C* ssDNA was incubated on gold films without the anchor probe being present was also undertaken to determine the extent of non-specific binding. As Fig. 4 shows, no luminescence signal was measured when the highest concentration of target DNA is used in the control experiment. This implies that the non-specific binding is minimal across the range of concentrations of target DNA. It is important to note that the room temperature assays in whole blood were not undertaken due to the coagulation of whole blood at room temperature within 10–15 min as shown previously (Aslan et al., 2007).

We employed a computational electrodynamics modeling technique, called FDTD, to predict the interactions of microwaves with our sample geometry. In a standard microwave cavity, the incident microwave field interacts with the three dimensional sample geometry. For gold disk 5 mm in diameter rotating in a microwave cavity the electric field is approximated to be constant in the z-dimension. Using FDTD simulation software, we simulated 2.45 GHz microwave field incident on a gold disk with a diameter of 5 mm and 5 um thickness and calculated the respective electric field distributions (Fig. 5A). The scale bars (blue to red) on the side of the graph represent the effectiveness of heating of the wells (via electric field distribution) with the blue color corresponding to minimal heating and red representing maximal heating. As the FDTD simulation shows, the electric field distribution from a microwave field incident on a 5 mm gold disk is highest in proximity to the edge of the disk (Fig. 5A—Left). Since increased electric field distributions lend to substantial dielectric loss to materials with large dielectric constants (i.e. water), one would expect a rise in temperature in media proximal to the increased electric fields. Consequently, one would expect the maximum temperature increase to occur proximal to the gold disk to the localized microwave field, as demonstrated in a cartoon drawing in (Fig. 5A—Right). It is also important to note that the gold disk is rotating in the microwave cavity and all sides of the gold disk are expected to be exposed to microwaves *equally*.

Given the results of the above FDTD calculations for microwave heating of our sample geometry, we measured the change in temperature of the assay medium during microwave heating with a thermal imaging camera as shown in Fig. 5B. Due to the physical limitations in our microwave setup that was specifically modified for thermal imaging, we only acquire temperature data for the first 20 s. We note that the temperature data presented here provide an insight for microwave heating of samples for a longer period of time. Fig. 5B

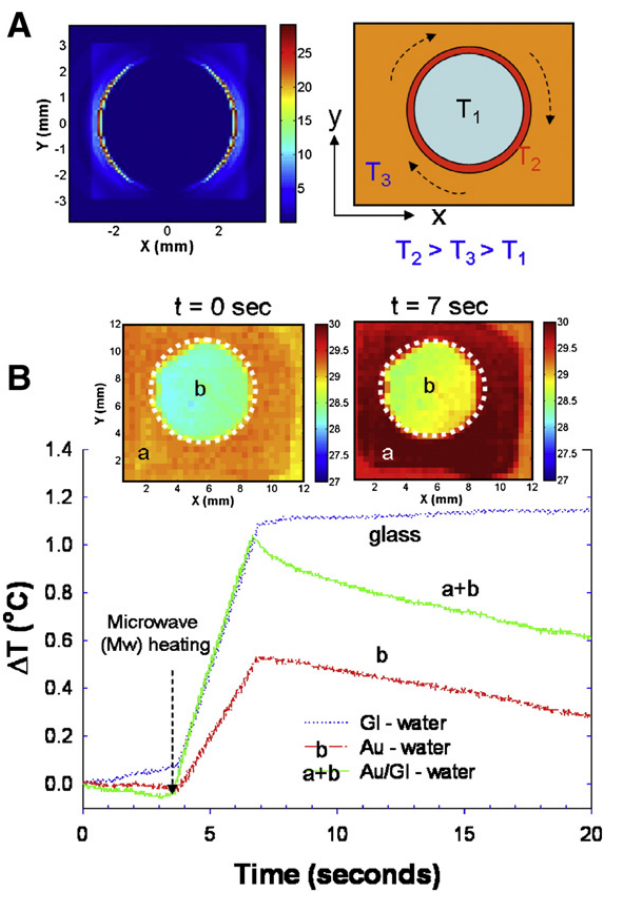


Fig. 5. Simulation and thermal imaging studies. (A) Left- Finite-Difference Time-Domain (FDTD) calculations for a gold disk of 5 mm diameter in a 2.45 GHz microwave field, Right — a cartoon drawing of the sample geometry: the sample rotates during the microwave heating. Temperature (T) distribution across the sample geometry is also shown. (B) The change in average temperature (ΔT) of the sample as captured using a thermal imaging camera during microwave heating. The insets show a snapshot of the thermal images of the sample geometry before ($t$=0 s) and during ($t$=7 s) microwave heating.

shows that the average temperature of the bulk solution on a plain glass surface (line *glass*) increases, but no subsequent cooling is observed during the remainder of the microwave heating process (Fig. 5B). In the presence of a gold disk (line $a+b$), the average temperature increase of the bulk solution is equivalent to that on plain glass, but an associated cooling is now evident (image region $a+b$). The average temperature increase for the bulk solution above *only* the gold disk (line $b$) is less than the temperature increase on glass and (Au/Glass) has a cooling rate similar to that of Au/glass. The inserts in Fig. 5B show the temperature distribution of water on our sample geometry before (time=0 s) and after (time=7 s) microwave heating captured using a thermal imaging camera.

We offer the following explanation for the increased DNA hybridization rates observed in this study based on the FDTD calculations and thermal imaging of the sample geometry during microwave heating: since the gold disk is preferentially heated around the edges and the temperature inside the gold disk is cooler than the edges, we believe that the buffer containing complementary target Hep C ssDNA above the gold disk moves from the warmer regions towards the cooler regions of the gold. As a result, this suggests that there is a larger influx of complementary DNA strands in the bulk

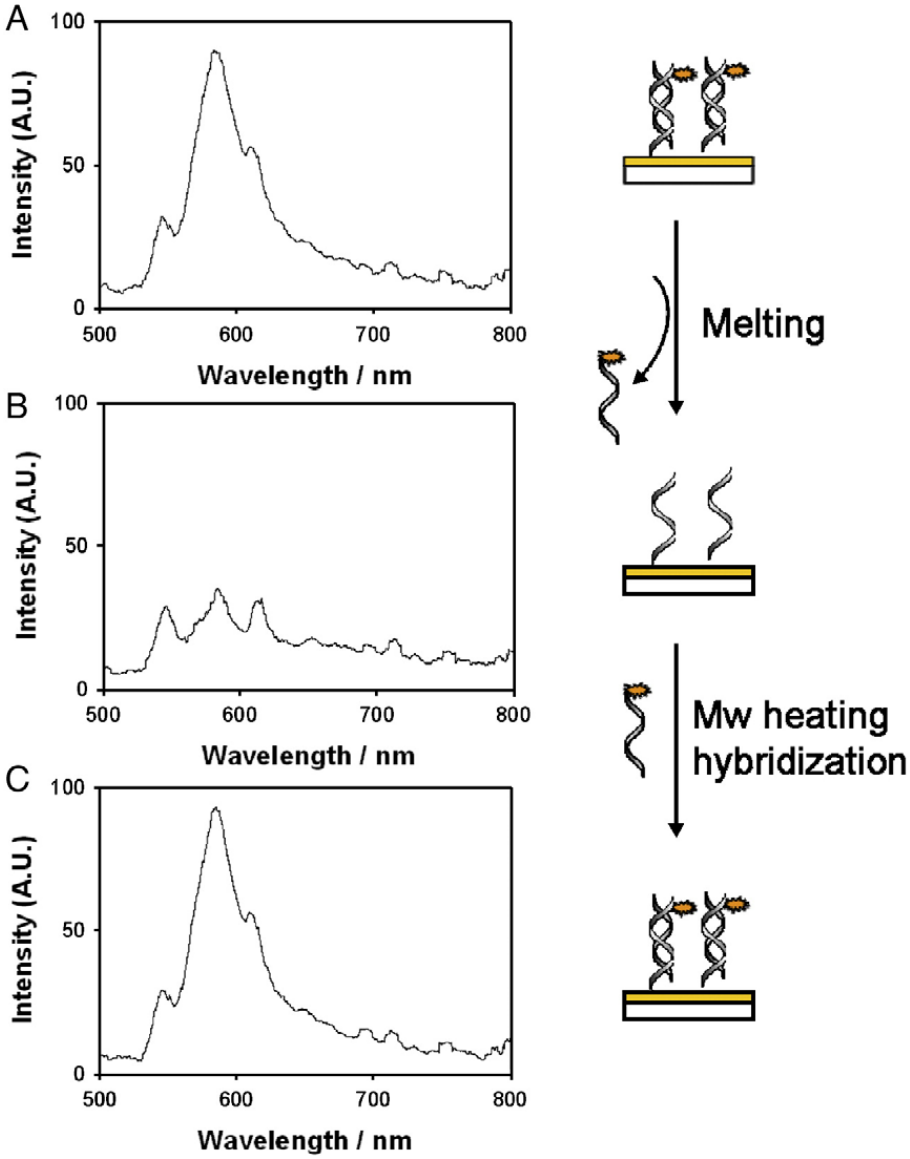


Fig. 6. Re-hybridization studies. Emission spectra of TAMRA-labeled target DNA (A) after hybridization, (B) after melting at 80 °C, and (C) after a further 20 s low power Mw heating with an additional 1000 nM TAMRA-labeled target DNA. The gold disk was washed with buffer several times between each measurement.

solution towards the anchor probe-modified gold disks where the localized temperature is cooler. In essence, this would explain the higher rate of binding and rapidity of the surface assays that we have previously described (Aslan and Geddes, 2005; Aslan et al., 2006, 2007).

In an earlier publication, we have shown that low power microwaves do not denature or perturb protein conformation (Aslan and Geddes, 2005). Similarly, we have considered the effects of low power microwave heating on the ability of ssDNA to re-hybridize with further complimentary target on the surface of gold films, which to the best of our knowledge has not been shown before. Fig. 6 shows the mission spectra of TAMRA-labeled target DNA after hybridization, after melting at 80 °C, and after a further 1 min low power microwave heating with an additional replenished 1000 nM TAMRA-labeled target ssDNA. The gold disk was washed with buffer several times between each measurement. As one can see, the TAMRA fluorescence intensity is retained after re-hybridization (Fig. 6B), which *strongly suggests* that the DNA is unaltered during low power microwave heating. This finding also suggests that future surfaces made for MA-SPCL based assays can be made both reusable and reversible for DNA target sensing applications.

### *3.1. Advantages of the MA-SPCL DNA hybridization platform technology*

The MA-SPCL-based DNA detection methodology described here has several notable advantages over current DNA detection technologies (Difilippantonio and Ried, 2003; Morrison, 2003), including:

1. DNA hybridization assays on gold films can be driven to completion within 1 min while retaining the same sensitivity as room temperature hybridization assays, which can take up to many hours to reach completion.
2. The need for extra separation steps for DNA samples in whole blood is eliminated since whole blood samples can be used directly with the MA-SPCL technology. We also believe other complex biological media such as tears and saliva can be both studied and probed in our system.
3. The low power microwaves used here do not crosslink or perturb the DNA. Our studies have shown that assays can be made completely reversible when using low power microwaves for kinetic acceleration, providing for identical final fluorescence intensities after melting and re-hybridization, c.f. Fig. 6.
4. MA-SPCL affords for highly directional and polarized emission, which is typically more sensitive than conventional isotropic fluorescence emission based assays (Aslan et al., 2005).
5. We do not observe any dielectric breakdown of the small gold discs, the gold films (Whittaker and Mingos, 1993), but instead heat buffer at their periphery, due to the enhanced electric fields around them.
6. The gold films are thin, inexpensive and can be cast on a variety of substrates (Matveeva et al., 2005).
7. Since the luminescence emission couples to surface plasmons from distances up to 300 nm in the reverse Kretschmann configuration (Gryczynski et al., 2004), MA-SPCL is a unique technology for the detection of target DNA with potentially >100 nucleotides.

## 4. Conclusions

In conclusion, the application of a previously described technique, Microwave-Accelerated Surface Plasmon-Coupled Luminescence (MA-SPCL) technique to fast and sensitive DNA hybridization assays in buffer and whole blood is presented. In this technique, while microwave heating affords the hybridization assay to be completed <1 min, surface plasmon-coupled luminescence additionally offers the detection of directional and polarized plasmon-coupled luminescence through the back a gold film at a certain angle. A fluorophore-labeled target ssDNA specific to *Hepatitis C* was hybridized with an anchor probe with a complementary strand on the gold film for 1 min in a microwave cavity or for 4 h at room temperature, which corresponds to up to 240-fold reduction in the assay run time explained by the temperature differential created on the surface of the gold disks by microwave heating. Control experiments, run in the absence of an anchor from the gold films, yielded no luminescence emission up to 1000 nM target oligonucleotide while a slight emission was observed for a target oligonucleotide concentration of 5000 nM. It was also shown again by our group that low power microwave heating reduces the extent of non-specific binding of target ssDNA, which affords for a further increase in sensitivity of the MA-SPCL-based DNA hybridization assays. FDTD calculations and thermal imaging experiments also provided an insight into the interactions of microwaves with the sample geometry to further our understanding of microwave-accelerated plasmonics effects for ultra fast surface DNA hybridization assays.

In closing, the MA-SPCL technique offers an alternative approach to current DNA based detection

technologies, especially when speed and sensitivity is required, such as in the identification of DNA or even RNA-based diseases that affect human health. Work is currently underway in our laboratories in this regard and will be reported in due course.

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## References

Aslan, K., Geddes, C.D., 2005. Microwave-accelerated metal-enhanced fluorescence: platform technology for ultrafast and ultrabright assays. Analytical Chemistry 77, 8057.

Aslan, K., Gryczynski, I., Malicka, J., Matveeva, E., Lakowicz, J.R., Geddes, C.D., 2005. Metal-enhanced fluorescence: an emerging tool in biotechnology. Current Opinion in Biotechnology 16, 55.

Aslan, K., Holley, P., Geddes, C.D., 2006. Microwave-Accelerated Metal-Enhanced Fluorescence (MAMEF) with silver colloids in 96-well plates: application to ultra fast and sensitive immunoassays, high throughput screening and drug discovery. Journal of Immunological Methods 312, 137.

Aslan, K., Malyn, S.N., Geddes, C.D., 2007. Microwave-accelerated surface plasmon-coupled directional luminescence: application to fast and sensitive assays in buffer, human serum and whole blood. Journal of Immunological Methods 323, 55.

Brown, P.O., Botstein, D., 1999. Exploring the new world of the genome with DNA microarrays. Nature Genetics 21, 33.

Difilippantonio, M., Ried, T., 2003. Technicolor genome analysis. In: Lakowicz, J.R. (Ed.), Topics in Fluorescence Spectroscopy, vol. 7. Kluwer Academic Publishers/Plenum Press, New York, p. 291.

Ekgasit, S., Stengel, G., Knoll, W., 2004. Concentration of dye-labeled nucleotides incorporated into DNA determined by surface plasmon resonance-surface plasmon fluorescence spectroscopy. Analytical Chemistry 76, 4747.

Gryczynski, I., Malicka, J., Nowaczyk, K., Gryczynski, Z., Lakowicz, J.R., 2004. Effects of sample thickness on the optical properties of surface plasmon-coupled emission. Journal of Physical Chemistry B 108, 12073.

Iwabuchi, K., Kubota, T., Kashiwa, T., 1996. Analysis of electromagnetic fields in a mass-produced microwave oven using the finite-difference time-domain method. Journal of Microwave Power and Electromagnetic Energy 31, 188.

Kessler, H.H., 2007. Design and work-up of a new molecular diagnostic assay based on real-time PCR. Methods in Molecular Biology 353, 227.

Komurian-Pradel, F., Paranhos-Baccala, G., Sodoyer, M., Chevallier, P., Mandrand, B., Lotteau, V., Andre, P., 2001. Quantitation of HCV RNA using real-time PCR and fluorimetry. Journal of Virological Methods 95, 111.

Lakowicz, J.R., 2004. Radiative decay engineering 3. Surface plasmon-coupled directional emission. Analytical Biochemistry 324, 153.

Liebermann, T., Knoll, W., 2000. Surface-plasmon field-enhanced fluorescence spectroscopy. Colloids and Surfaces A-Physicochemical and Engineering Aspects 171, 115.

Liebermann, T., Knoll, W., Sluka, P., Herrmann, R., 2000. Complement hybridization from solution to surface-attached probe-oligonucleotides observed by surface-plasmon-field-enhanced fluorescence spectroscopy. Colloids and Surfaces A-Physicochemical and Engineering Aspects 169, 337.

Lumerical Solutions, I. (Commercial Software), 2006. FDTD Solutions Manual Release 4.0. Vancouver, BC.

Matveeva, E.G., Gryczynski, I., Malicka, J., Gryczynski, Z., Goldys, E., Howe, J., Berndt, K.W., Lakowicz, J.R., 2005. Plastic versus glass support for an immunoassay on metal-coated surfaces in optically dense samples utilizing directional surface plasmon-coupled emission. Journal of Fluorescence 15, 865.

Morrison, L., 2003. Fluorescence in nucleic acid hybridization assays. In: Lakowicz, J.R. (Ed.), Topics in Fluorescence Spectroscopy, vol. 7. Kluwer Academic Publishers/Plenum Press, New York, p. 69.

Previte, M.J., Geddes, C.D., 2007. Microwave-triggered chemiluminescence with planar geometrical aluminum substrates: theory, simulation and experiment. Journal of Fluorescence 17, 279.

Stelzl, E., van der Meer, C., Gouw, R., Beld, M., Grahovac, M., Marth, E., Kessler, H.H., 2007. Determination of the *hepatitis C* virus subtype: comparison of sequencing and reverse hybridization assays. Clinical Chemistry and Laboratory Medicine 45, 167.

Stengel, G., Knoll, W., 2005. Surface plasmon field-enhanced fluorescence spectroscopy studies of primer extension reactions. Nucleic Acids Research 33, e69.

Stuyver, L., Wyseur, A., van Arnhem, W., Hernandez, F., Maertens, G., 1996. Second-generation line probe assay for *hepatitis C* virus genotyping. Journal of Clinical Microbiology 34, 2259.

Suckling, J.R., Hibbins, A.P., Lockyear, M.J., Preist, T.W., Sambles, J.R., Lawrence, C.R., 2004. Finite conductance governs the resonance transmission of thin metal slits at microwave frequencies. Physical Review Letters 92.

Suckling, J.R., Sambles, J.R., Lawrence, C.R., 2007. Resonant transmission of microwaves through a hexagonal array of holes in a thin metal layer. New Journal of Physics 9.

Tawa, K., Yao, D., Knoll, W., 2005. Matching base-pair number dependence of the kinetics of DNA–DNA hybridization studied by surface plasmon fluorescence spectroscopy. Biosensors & Bioelectronics 21, 322.

Walker, N.J., 2002. Tech.Sight. A technique whose time has come. Science 296, 557.

Whittaker, A.G., Mingos, D.M.P., 1993. Microwave-assisted solid-state reactions involving metal powders and gases. Journal of the Chemical Society-Dalton Transactions 2541.