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Research paper

Microwave-Accelerated Surface Plasmon-Coupled Directional Luminescence: Application to fast and sensitive assays in buffer, human serum and whole blood

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Abstract

The applicability of a new technique, Microwave-Accelerated Surface Plasmon-Coupled Luminescence (MA-SPCL) for fast and sensitive bioassays in buffer, serum and whole blood using quantum dots as luminescence reporters is demonstrated. In this regard, a model bioassay based on the well-known interactions of biotin and streptavidin is used. Using MA-SPCL, the bioassay was kinetically completed within 1 min with the use of low power microwave heating as compared to the identical bioassay which took in excess of 30 min to reach >95% completion at room temperature, a 30-fold increase in assay kinetics. The luminescence emission from the quantum dots was coupled to surface plasmons of the gold film, enabling the detection of the luminescence emission in a highly directional fashion as compared to the normal isotropic emission, for enhanced sensitivity and detection.

The combined effect of microwaves for faster assay kinetics, with surface plasmon-coupled luminescence for sensitive luminescence measurements, has also made possible the demonstration of the use of the MA-SPCL technique for assays run in complex media, such as human serum and whole blood, where the same assay could not be performed at room temperature due to the coagulation of blood. In the MA-SPCL assay run in serum and whole blood, the luminescence intensity from 33 nM quantum dots was 75% and 20% that of the luminescence intensity from the assay run in buffer, with a signal to noise ratio of 12.5 and 3, respectively.

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Keywords: Microwave-Accelerated Metal-Enhanced Fluorescence; Metal-Enhanced Fluorescence; Surface Plasmon Fluorescence Spectroscopy; Surface plasmon-coupled emission; Low power microwaves; Ultra-fast assay kinetics

Abbreviations: BSA, Bovine Serum Albumin; MAMEF, Microwave-Accelerated Metal-Enhanced Fluorescence; MEC, Metal-Enhanced Chemiluminescence; MEF, Metal-Enhanced Fluorescence; MA-SPCL, Microwave-Accelerated Surface Plasmon-Coupled Directional Luminescence; RPM, Radiating Plasmon Model; SPCE, Surface Plasmon-Coupled Emission; SPR, Surface Plasmon Resonance; SPFS, Surface Plasmon Fluorescence Spectroscopy.

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1. Introduction

Fluorescence spectroscopy is one of the most widely used detection techniques in medical diagnostics (Borrebaeck, 2000; Vo-Dinh et al., 1993; Hemmila, 1992). However, the fluorescence signal of interest is often challenged by high background luminescence affecting the sensitivity of fluorescence-based medical diagnostic tests. Several approaches have been suggested to minimize the background signal caused by the sample matrix (e.g., serum or whole blood in clinical diagnostics), including fluorescence/polarization kinetics detection (Gomez-Hens and Aguilar-Caballos, 2003), time-gated detection based on long-lived lanthanide emission (Lövgren and Pettersson, 1990), and two-photon excitation (Baker et al., 2000). Due to the high fluorescence background and optical density, only a small number of immunoassays are usually carried out in whole unseparated blood, except in some cases where additional washing steps are performed before the output signal is measured (Choi et al., 2004; von Lode et al., 2004; Tarkkinen et al., 2002). Therefore, there is a continuous and unequivocal need for the development of better reporter molecules and/or detection methods for the direct analysis of whole blood samples.

Semiconductor quantum dots (QDs) have been introduced as an alternative to fluorophores for optical imaging and spectroscopy (Chan and Nie, 1998; Dubertret et al., 2002; Akerman et al., 2002). QDs are nanometer-sized inorganic structures with physical dimensions smaller than the exciton Bohr radius. QDs exhibit unique luminescence emission characteristics by changing their size or composition. As fluorescent probes, QDs have several advantages over conventional organic fluorophores. Their emission spectra are narrow, symmetrical, and tunable according to their size and material composition, allowing closer spacing of different probes without substantial spectral overlap. Moreover, they exhibit excellent stability against photobleaching. Most significantly, they display broad absorption spectra, making it possible to excite all QDs simultaneously at a single excitation wavelength (Bruchez et al., 1998; Chan et al., 2002).

After its introduction in the early 1990s, Surface Plasmon Resonance (SPR) spectroscopy has become a widely used technique for the monitoring of biological binding events. Despite its widespread use, SPR still has limited sensitivity when it comes to differentiating between large and small biomolecules in the same assay. In addition, its lack of applicability for multiple detections at one surface is insufficient for diagnostic medical applications. In this regard, recently, many efforts were focused on the enhancement of the sensitivity of the SPR technique especially by combining it with fluorescence spectroscopy (Neumann et al., 2002; Schutt et al., 2003). This new technique was first named Surface Plasmon Fluorescence Spectroscopy (SPFS) (Kambhampati et al., 2001; Liebermann and Knoll, 2000; Liebermann et al., 2000), and now is also called Surface Plasmon-Coupled Emission (SPCE) (Lakowicz, 2004). In SPFS or SPCE, the resonant excitation of an evanescent surface plasmon mode can be used to excite the fluorophores, chemically attached to the target biomolecules. Upon the binding of fluorophore-labeled target biomolecules to their binding partners (analytes) at the metal/solution interface, the chromophore is exposed to the strong optical fields (that can be obtained in PSP resonance) giving rise to significant enhancement factors. Then, the emitted fluorescence photons are monitored, which is correlated to the concentration of the analyte. The use of fluorescence detection schemes in combination with the resonant excitation of surface plasmons has been shown to increase the sensitivity for bioanalyte monitoring considerably (Liebermann and Knoll, 2000).

In 2004, Robelek et al. (2004) showed the feasibility of the use of the SPFS technique in combination with surface plasmon enhanced fluorescence microscopy (SPFM) with quantum dot QD–DNA conjugates on gold films. The use of these two techniques resulted in the simultaneous qualitative analysis of QD-conjugated analyte DNA strands as multicolor images. In this regard, SPFS allowed the monitoring of the binding event in terms of fluorescence intensity (maximum at 62.5°) and emission spectrum. In a later paper, Lakowicz et al. (Gryczynski et al., 2005) showed the SPCE of QDs in a 25 nm polymer film spin coated on a glass slide with 50 nm of silver and a 5-nm protective SiO_2 layer. In this paper, the surface plasmons emitted a hollow cone of radiation (due to symmetry conditions) into an attached hemispherical glass prism at a narrow angle of 48.5° when the quantum dots were excited at 514 nm. It was shown that the directional radiation (SPCE) preserved the spectral properties of QD emission and was highly p-polarized irrespective of the excitation polarization.

The development of the above mentioned new techniques in combination with the use of highly photostable quantum dots addresses the issue of bioassay sensitivity. However, most biological recognition events, such as the antigen–antibody interactions, DNA hybridization, are most often kinetically very slow, requiring long incubation times, very few assays

subsequently being complete in less than 10 min (Bange et al., 2005; Hemmila, 1992; Van Dyke and Van Dyke, 1990; Ozinkas, 1994). In this regard, a new platform technology, Microwave-Accelerated Metal-Enhanced Fluorescence (MAMEF) which couples the benefits of Metal-Enhanced Fluorescence (a phenomenon which significantly increased the fluorescence signals of fluorophores in close proximity (< 10 nm) to metallic nanostructures) with the use of low power microwaves to kinetically accelerate bioaffinity reactions was recently introduced by us for bioassays (Aslan and Geddes, 2005), immunoassays (Aslan and Geddes, 2006) and DNA hybridization (Aslan et al., 2006). In the MAMEF technology, the MEF phenomenon allows for much more sensitive assays to be developed, combined with low power microwave heating to kinetically accelerate assays to completion within seconds, significantly reducing a bioassays' run time. The much faster kinetics in MAMEF assays is made possible by the employment of low power microwaves in combination with the presence of the silver nanoparticles. A detailed explanation of the effects of microwaves in conjunction with silver nanoparticles on the kinetics of biomolecular interactions, such as protein–protein and antibody–antigen interactions, has been given elsewhere (Aslan and Geddes, 2005). In short, when a polar medium (like the assay buffer) and non-continuous silver nanoparticles are exposed to microwave radiation (≈3 GHz) the charge carriers in the metal, which are displaced by the electric field, are subjected to resistance in the medium in which they travel due to collisions with the lattice phonons (Whittaker and Mingos, 1993). This leads to localized heating around the silver nanostructures in addition to the heated solvent, rapidly accelerating assay kinetics. Fluorescence lifetime and fluorescence resonance energy transfer (FRET) studies have also shown that microwave heating does not induce protein structural (denaturation) or environmental changes (Aslan and Geddes, 2005). Therefore, MAMEF provides for ultra-fast and ultra-bright immunoassays to be realized.

In this paper, the application of microwave-accelerated technology for fast and sensitive bioassays in human serum and whole blood based on surface plasmon-coupled directional luminescence, MA-SPCL, is presented. To demonstrate the applicability of the MA-SPCL technique, a model bioassay based on biotin–streptavidin interactions was constructed on a 50 nm thick gold film. In this regard, quantum dots (streptavidin conjugated) were used as reporter molecules, where the surface plasmon-coupled directional luminescence intensity is measured and correlated to the amount of streptavidin (10–1250 nM) 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.

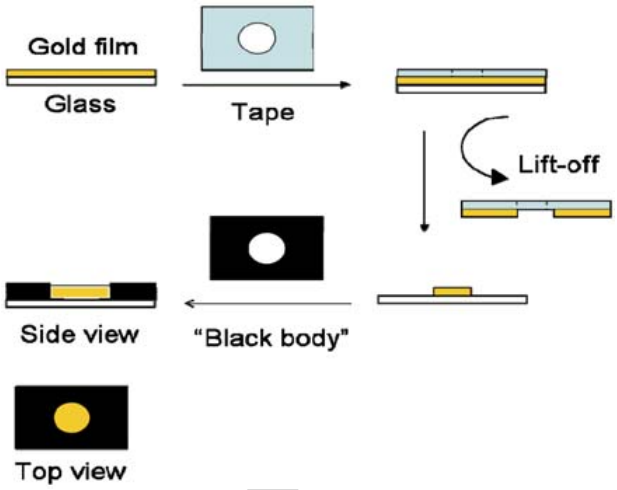

Fig. 1. Schematic representation of producing gold-coated glass slides (gold disks) with a 5 mm gold spot for MA-SPCL bioassays.

2. Materials and methods

2.1. Materials

Bovine–biotinamidocaproyl-labeled Albumin (biotinlyated-BSA) and premium quality plain glass microscope slides (75 × 25 mm) were obtained from Sigma-Aldrich. Quantum dot 655-labeled streptavidin (Qdots-streptavidin) was obtained from Molecular Probes (Eugene, OR). All chemicals were used as received.

2.2. Methods

2.2.1. Preparation of the metal films for Surface Plasmon-Coupled Luminescence (SPCL) Spectroscopy

Firstly, the plain glass slide was coated with a 50 nm thick gold film by vapor deposition (EMF Corp., Ithaca, NY). Then, a tape with a 5 mm circular disk is applied onto the gold film and was lifted off the surface that resulted in the removal of the gold film except a 5 mm circular disk (Fig. 1). Finally, a black electrical tape that is attached to a self-sticking paper, containing a 5 mm wide circular disk (referred to as a "black body") was attached on the gold-coated glass slide while the two circular disks matched, prior to the assay fabrication and subsequent SPCL experiments.

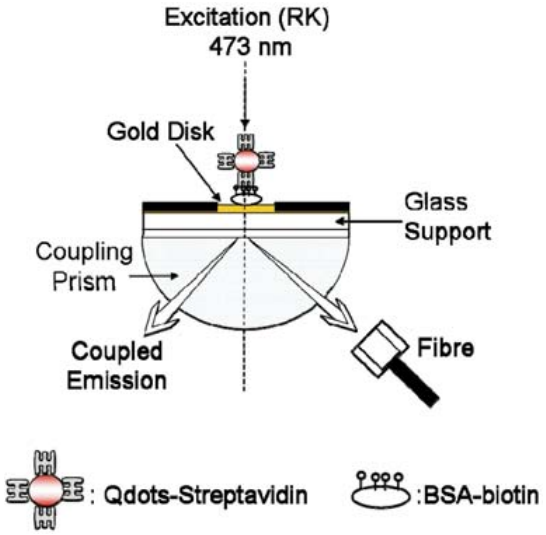

Fig. 2. Optical setup for Microwave-Accelerated Surface Plasmon-Coupled Luminescence (MA-SPCL) bioassays. The sample is excited directly in the reverse Kretschmann (RK) configuration. The assay is undertaken on the gold-coated glass slide, which is attached to the glass prism with index matching fluid. The two arrows on the left side show the directional coupled luminescence emission. Figure is not drawn to scale.

2.2.2. Microwave-Accelerated SPCL assay

The model assay used in this paper is based on the well-known interactions of biotin and streptavidin. Biotin groups are introduced to the metal surface through biotinylated-BSA, which, similar to HSA, readily forms a monolayer on the surfaces of glass and noble metals (Green, 1975). Binding of the biotinylated-BSA to the metal film was accomplished by incubating 10 μM biotinylated-BSA solution in the "black body" micro cuvettes for 30 min, followed by rinsing with deionized water to remove the unbound material. For the model assay, then a 30 μl of varying concentrations of Qdots-streptavidin was subsequently added into the biotinylated-BSA coated metal film-coated micro cuvettes for 30 min for the control experiments at room temperature (20 °C), and 1 min in the microwave cavity (0.7 ft^3, GE Compact Microwave Model: JES735BF, max power 700 W). The power setting was set to 3 which corresponded to 210 W over the entire cavity. This power is similar to the numerous reports using low power microwaves for immunolabeling (Chicoine and Webster, 1998), immunostaining (Micheva et al., 2001; Petrali and Mills, 1998), in immunocytochemistry (Madden, 1998; Rangell and Keller, 2000; Schichnes et al., 1999) and histological microwave processing (Rassner et al., 1997; Schray et al., 2002). In all the experiments performed with low power microwaves, using metal film-coated micro cuvettes modified with the "black body", there was no evidence of surface drying. The coated slides were attached to a hemi-cylindrical prism made of BK7 glass with index matching fluid. This combined sample was positioned on a precise rotary stage that allows excitation and observation at any desired angle relative to the vertical axis along the cylinder. The sample was excited using the Reverse Kretschmann configuration (Fig. 2) from the air or sample side, which has a refractive index lower than the prism. The excitation was from the second harmonic (473 nm) of the diode- pumped Nd:YVO4 laser (compact laser pointer design, output power ≈30 mW) 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 488 nm super notch filter.

3. Results and discussion

In order for the gold-coated glass slides to be used in MA-SPCL bioassays, they have to withstand the

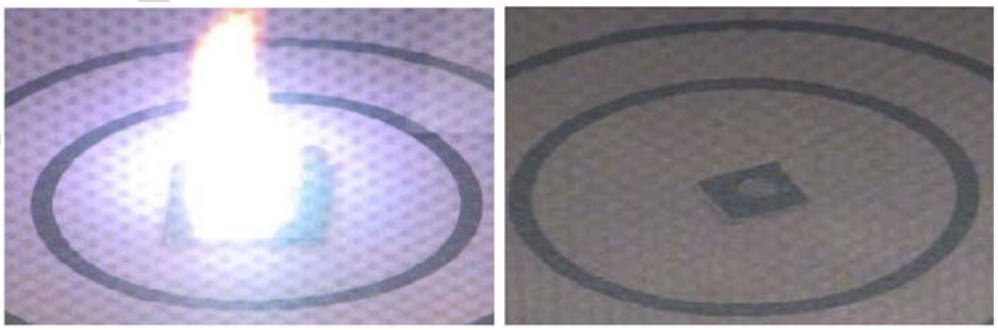

Fig. 3. Photographs of gold-coated glass slides during microwave heating. Left — The glass slide with a continuous gold coating (size $\approx > \lambda/10$), and right — the glass slide with a 5 mm diameter gold disk (size $\approx << \lambda/10$). While the glass slide with a continuous gold coating sparks and arcs within seconds, the glass slide featuring a small 5 mm diameter gold disk is not affected during 1 min of microwave heating.

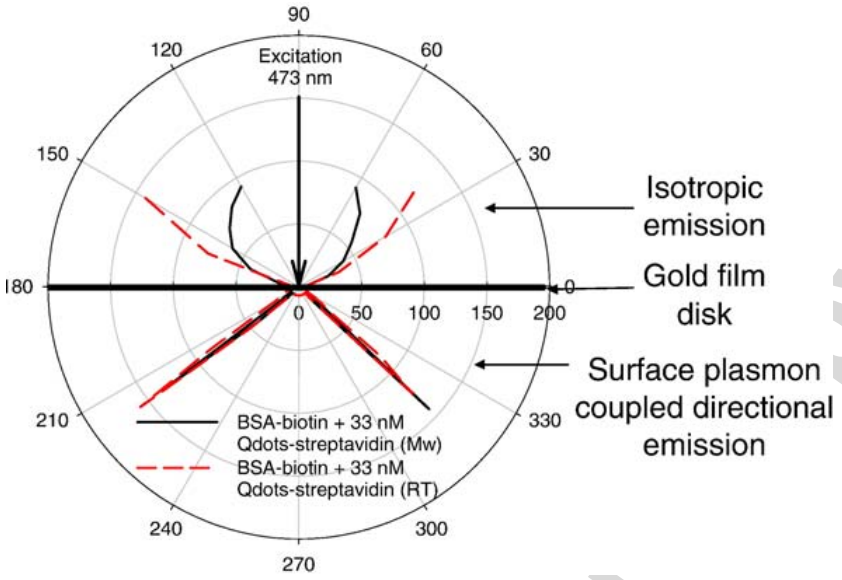

Fig. 4. Angular distribution of luminescence for 33 nM of quantum dots-streptavidin used in the MA-SPCL assay, both microwave heated (Mw) and run at room temperature (RT). Angular-axis: 0 to 360°, radial-axis: luminescence intensity in arbitrary units (A.U.). The background intensity (noise) was 6 A.U. See Fig. 2 for the experimental setup.

microwave heating while the bioassay is being driven to completion, as well as further physical treatments, such as multiple buffer washing steps. Thus, at first, the feasibility of the gold-coated glass slides, like those commonly used in Surface Plasmon Fluorescence Spectroscopy (Kambhampati et al., 2001; Liebermann and Knoll, 2000; Liebermann et al., 2000) and Surface Plasmon Resonance (Lofas et al., 1991) were tested for their use in MA-SPCL assays via exposure to microwaves. Fig. 3-left shows the photograph of typically used gold-coated glass slide during microwave heating. When exposed to microwaves, the glass slide with a continuous gold coating sparks and is destroyed within seconds, proving them not to be useful with MA-SPCL bioassays. However, the glass slide featuring only a 5 mm gold disk and a black body, made from the same gold-coated glass slide used in Fig. 3-left, is not destroyed even after continuous (1 min) and repetitive (5 times) microwave heating, Fig. 3-right. This was possible due to 1) the fact that the surface area of the gold disks is smaller than the area required for the surface charge buildup (Whittaker and Mingos, 1993) and eventual destruction of the gold coating, and 2) the black body absorbs an excess of the microwaves, providing additional protection for the gold coating. In the light of these observations, only the glass slide featuring a 5 mm gold disk can be used for MA- SPCL bioassays, a significant observation and finding, and attributed to the size of the disk being sub-wavelength with respect to the microwaves.

To demonstrate MA-SPCL bioassays on small disk gold films, we employed a model assay based on the well-known interactions of biotin and avidin (Wilchek and Bayer, 1990; Baziard et al., 1988). The model protein assay was constructed with biotinylated-BSA surface modified gold films and streptavidin-modified quantum dots, and run both at room temperature and microwave accelerated (heated). This model protein system affords for simple kinetics, i.e. no back reactions are expected due to the strong association of biotin and avidin (Wilchek and Bayer, 1990; Baziard et al., 1988). Fig. 4 shows the angular distribution of luminescence for 33 nM of quantum dots used in the MA-SPCL assay, both microwave heated (Mw, 1 min) and run at room temperature (RT, 30 min).

The surface plasmon-coupled luminescence (observed through a prism between the angles of 180 and 360°, see Fig. 4 for the experimental setup) is of similar intensity and is highly directional at two angles, 217 and 323°, for MA-SPCL assays, both microwave heated and run at room temperature, Fig. 4. This indicates that the biotin–avidin interactions, which took place in 30 min at room temperature, were completed within 1 min using microwave heating, corresponding to a 30-fold increase in kinetics due to microwave heating. The experiments showed that at least 1 min of microwave heating was required for the kinetics of biotin–avidin interactions on the gold film to be completed in the MA-SPCL technique (data not shown), where the completion of the assay kinetics was evaluated by

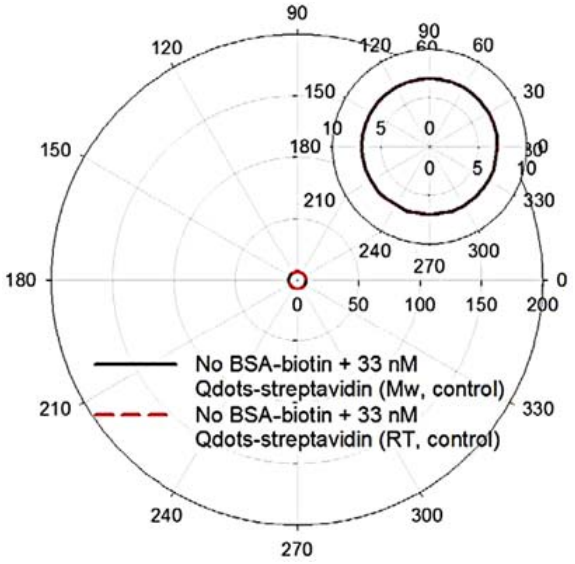

Fig. 5. Angular distribution of luminescence for 33 nM of quantum dots-streptavidin used in the control MA-SPCL assay (Mw, control) and at room temperature (RT, control). The control assay is undertaken by omitting one of the binding partners, i.e., BSA-biotin. The inset shows an enlarged region. Note that this figure is drawn to the same scale as Fig. 4. Angular-axis: 0 to 360°, radial-axis: luminescence intensity in arbitrary units (A.U.). The background intensity (noise) was ≈6 A.U.

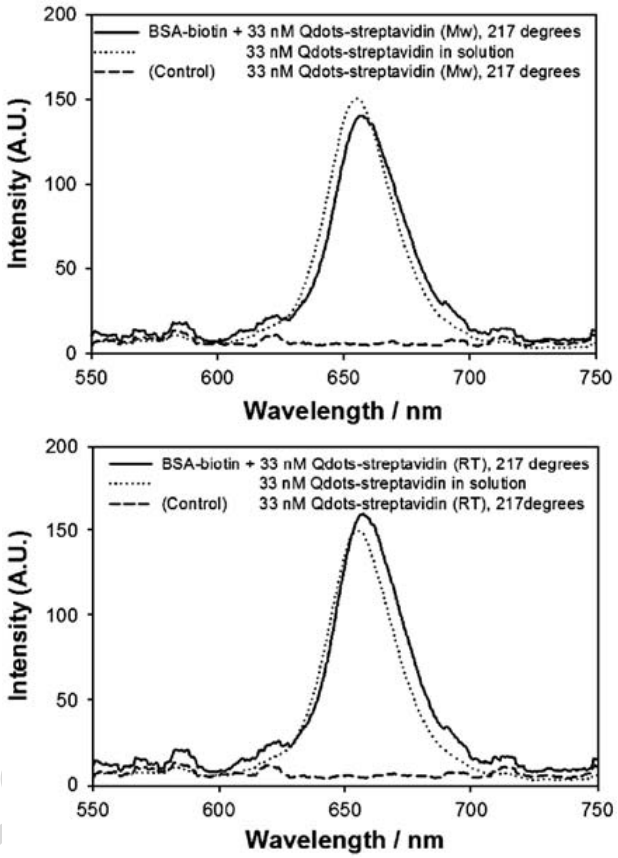

Fig. 6. The emission spectra for 33 nM quantum dots used in the MA-SPCL assay and the control MA-SPCL assay, (Mw) and (control, Mw), respectively (top). The emission spectra of the assay run at room temperature and the control assay at room temperature, (RT) and (control, RT), respectively (bottom). All SPCL spectra were recorded at 217° (the directional emission angle) as both indicated in Fig. 1 and shown in Fig. 4. The emission spectrum from quantum dots in solution (excited with 473 nm laser) is also shown for good spectral comparison. A.U. — arbitrary units.

comparing the signal from the same assay at room temperature. The free-space luminescence (between the angles of 0 and 180°) for MA-SPCL assays both microwave heated and at room temperature, are isotropic and are similar to the intensity observed on the prism side (217 or 323°).

A control assay, where one of the binding partners, i.e., BSA-biotin is omitted, was also undertaken both at room temperature (RT) and with microwave heating (Mw) to determine the extent of non-specific binding to the surface of the gold films. Fig. 5 shows the angular distribution of luminescence for 33 nM of quantum dots used in the control MA-SPCL assay. As can be seen from Fig. 5, the luminescence intensity was similar to the background intensity both at room temperature and the MA-SPCL assay, which indicated there is very little non-specific binding of the quantum dots-streptavidin when the biotinylated-BSA was not present on the surface.

Fig. 6 shows the emission spectra, for 33 nM quantum dots-streptavidin used in the MA-SPCL assay and the control MA-SPCL assay, the identical assay run at room temperature and the control assay at room temperature, which were recorded at 217° in Fig. 4, as well as the emission spectrum from 33 nM quantum dots in solution, shown for spectral comparison. The assay yields a similar final luminescence intensity, after 1 min microwave heating (≈150 A.U.) as compared to a 30 minute room temperature incubation, c.f. Fig. 6 top and bottom, which is very similar to that obtained in solution. This demonstrates the utility of the MA-SPCL technique and that ultra-fast directional luminescence assays can be realized. Control experiments did not yield any signal since quantum dots-streptavidin could not bind to the surface due to the absence of biotinylated-BSA on the surface.

In order to demonstrate the utility of MA-SPCL assay in a quantitative manner, the MA-SPCL assay, which was undertaken with 33 nM quantum dots-streptavidin in Fig. 6, was repeated with varying concentrations of quantum dots-streptavidin, 10–1250 nM. Fig. 7-top shows the emission spectra of varying concentrations for the quantum dots used in the MA- SPCL assay measured at 217°, as well as the real-color photograph obtained through the same emission filter used in the experiments. The calibration curve (intensity at 665 nm

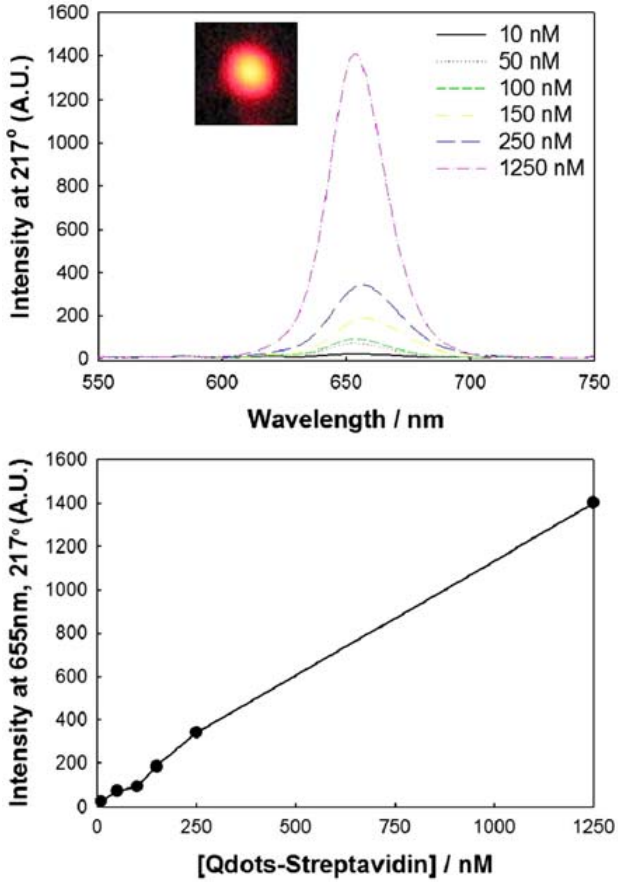

Fig. 7. The emission spectra of varying concentrations of quantum dots used in the MA-SPCL assay measured at 217° (top). The calibration curve, intensity at 665 nm vs. concentration of the quantum dots, is obtained from the top figure (bottom). The real-color photograph is obtained from the MA-SPCL assay undertaken with 50 nM quantum dots– streptavidin at 217°, the directional emission angle. A.U. — Arbitrary units. (For interpretation of the references to curves in color in this figure legend, the reader is referred to the web version of this article.)

vs. concentration of the quantum dots) is obtained from the Fig. 7-Top, and is shown in Fig. 7- Bottom. As can be seen from Fig. 7, the intensity measured at 655 nm and 217° in the MA-SPCL setup increases with the increasing concentration of quantum dots-streptavidin (Fig. 7-Top), and followed a linear trend within the range of quantum dots-streptavidin concentration used (Fig. 7-top). A real-color photograph taken through an emission filter at 217° is also given as a visual evidence for the surface plasmon-coupled luminescence.

Fig. 8 shows the time-dependent emission intensity at 665 nm for 33 nM of quantum dots used in the MA-SPCL assay, measured at 217°, the directional emission angle. The emission intensity was ≈ constant during 10 min of continuous excitation of the quantum dots on the surface. This indicates that quantum dots did not photobleach or undergo other physical changes that would alter the luminescence intensity, allowing the luminescence measurements to be performed for at least up to 10 min after the assay is completed. We do however notice a very slight increase in intensity over time. This has been attributed to the laser-induced drying of the sample during collection time. Although quantum dots are known to not photobleach as much as their fluorescent counterpart, this experiment was undertaken to emphasize the importance of evaluating the photostability of the luminescent probe used in the assay, especially when luminescent probes other than quantum dots are to be used.

Until now we have shown, the MA-SPCL assay undertaken in synthetic media, where the assay components were dissolved/dispersed in phosphate buffer pH 7.0. To investigate the applicability of the new MA-SPCL technique to assays in more complex media, such as serum or even whole blood, the MA-SPCL assays using quantum dots-streptavidin were repeated in buffer, serum and whole blood. Fig. 8-top shows the emission spectra for 33 nM quantum dots used in the MA-SPCL assay measured at 217° in different media. The peak intensity from the assay using quantum dots (at 655 nm) in serum was 75% of that peak intensity in buffer, indicating that the MA-SPCL assays can be easily done in serum with very large signal to noise ratios, 12.5 (75/6). Interestingly, the MA-SPCL assay using quantum dots in whole blood still yielded a measurable signal (20% that of MA-SPCL assay in buffer) with a signal to noise ratio of approximately 3, given that the S/N > 3 for fluorescence-based assays is considered acceptable (Lakowicz, 1999).

To further demonstrate the benefits of the MA-SPCL technique for whole blood assays, the assay using quantum dots-streptavidin was repeated in whole blood

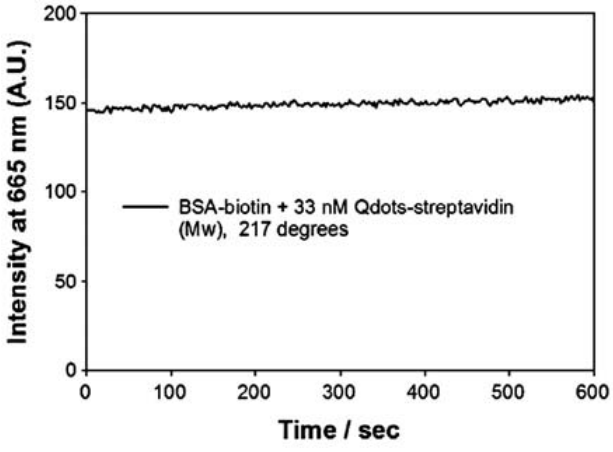

Fig. 8. The time-dependent emission intensity at 665 nm for 33 nM of quantum dots used in the MA-SPCL assay, measured at 217°, the directional emission angle. A.U. — arbitrary units.

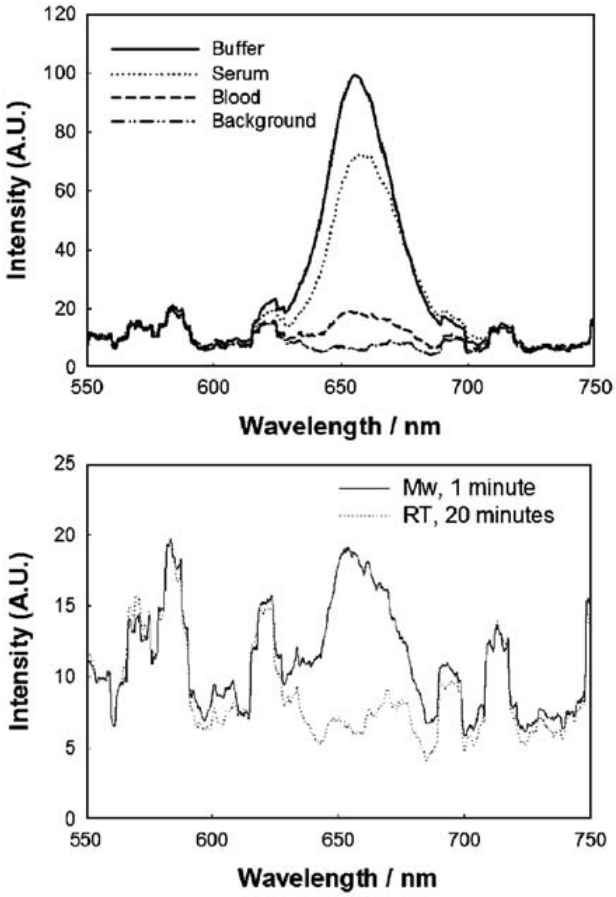

Fig. 9. The emission spectra for 33 nM quantum dots used in the MA-SPCL assay measured at 217° in different media (top). The background signal is obtained from the sample that only contains biotinylated-BSA (quantum dots not used) and was ≈6 A.U. The peak intensity obtained from the assay in buffer is normalized to 100 A.U. (the intensities from assays in serum and whole blood and the background are adjusted relative to intensity in buffer) for an easy comparison of the luminescent signal in percentage terms. The MA-SPCL assay (Mw, 1 min) and the SPCL assay at room temperature (RT, 20 min) performed in whole blood measured at 217° (bottom). A.U. — arbitrary units.

both with microwave heating and at room temperature for comparison. Fig. 9-bottom shows the MA-SPCL assay (Mw, 1 min) and the SPCL assay at room temperature (RT, 20 min) performed in whole blood measured at 217°. While the assay that was microwave heated (accelerated) for 1 min yielded a measurable signal, the assay that was undertaken at room temperature for 20 min did not yield any signal. This is due to the fact that the whole blood used in the assay at room temperature coagulated within 10 to 15 min, entrapping the quantum dots, resulting in no luminescence signal change. On the other hand, when the whole blood is microwaved for 1 min, significantly less coagulation of the blood occurred enabling the assay to be completed, which is a significant benefit to our approach to whole blood assays. It is important to note that the concentration of the quantum dots (33 nM) used in the whole blood MA-SPCL assay, was closer to the lower detection limit found here (Fig. 7), promising the use of the MA-SPCL assay in whole blood for a concentration range similar to that of the assay in buffer. This is made possible by the use of microwave heating that significantly reduces the assay time, so that the assay could be carried out in whole blood before the blood coagulates, while also decreasing the non-specific interactions. In this regard, numerous reports have employed microwaves and whole blood (Hirsch et al., 2003; Herron et al., 1997), strongly suggesting that whole blood components are not damaged by microwave exposure.

4. Conclusions

A new technique, Microwave-Accelerated Surface Plasmon-Coupled Luminescence (MA-SPCL) for fast and sensitive assays in buffer, serum and whole blood is presented. In this regard, a 50 nm thick gold disk, 5 mm in diameter, with a black body is used as the substrate for the MA-SPCL assays. It was found that the gold disk with the black body withstood the microwave exposures for up to 5 min enabling the completion of the assay, while the larger-sized 50 nm continuous gold film, like those used for typical SPR, sparked, arced and was destroyed within 10 s. In addition, the gold disk with the black body retained its physical properties after multiple assay washes. The feasibility of the MA-SPCL technique was demonstrated with a model assay using streptavidin conjugated quantum dots and surface-bound biotinylated-BSA. Control experiments were also undertaken to determine the extent of the non-specific interactions. With the MA-SCPL technique, 10–1250 nM of streptavidin was detected within 1 min, which corresponds to ≈30-fold faster kinetics as compared to the assay undertaken at room temperature. The accelerated assay kinetics were made possible by the heating of gold disk by microwaves, resulting in a slight increase in the bulk solution temperature, with higher temperature jumps close to the gold surface. Moreover, highly directional coupled luminescence emission (at 655 nm, 217° through the coupling prism) from quantum dots enabled the realization of sensitive luminescence measurements. The combined effect of microwaves for faster assay kinetics with surface plasmon-coupled luminescence for sensitive measurements also made possible the demonstration of the use of MA-SPCL technique for assays to be run in complex media such as human serum and whole blood, where the same assay

could not be performed at room temperature due to the coagulation of blood. In the MA-SPCL assay run in serum and whole blood, the luminescence intensity from 33 nM quantum dots was 75% and 20% that of the luminescence intensity from the assay run in buffer, with a signal to noise ratio of 12.5 and 3, respectively.

These findings suggests that highly sensitive and ultra-fast luminescence assays can realized in synthetic media as well as more complex media. To the best of our knowledge this is the first report on Microwave-Accelerated Surface Plasmon-Coupled Luminescence on metallic films and further studies using different metals and microwave frequencies are in progress in our laboratory, and will be reported in due course.

Finally, it is important to comment on the feasibility of the MA-SPCL method as a new generic research tool for other researchers in the immunoassay and immunology disciplines. The MA-SPCL technology requires researchers to combine standard, commercially available optics and detectors, with standard biochemical procedures, potentially being performed by any researcher. It is also possible that the MA-SPCL technique could be used in conjunction with the commercially available SPR instrument and technique. This could be accomplished by slight modifications to the size of the gold coating on the chip, to accommodate microwave heating and field distributions. Further studies are also underway in this regard and will be reported in due course.

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