Plasmonics (2008) 3:89–101
DOI 10.1007/s11468-008-9059-x

A Review of an Ultrafast and Sensitive Bioassay Platform Technology: Microwave-accelerated Metal-enhanced Fluorescence

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Received: 9 April 2008 / Accepted: 26 June 2008 / Published online: 17 July 2008

Abstract Since the publication of our first paper on the microwave-accelerated metal-enhanced fluorescence (MAMEF) bioassay platform technology in 2005 (Aslan and Geddes, Anal Chem 77:8057–8067, 2005), we have been repeatedly asked to comment on the advantages of "microwave heating" with plasmonic nanostructures over conventional heating for bioassays by many of our colleagues in the community. We note that one can find a couple of review articles, one by Mingos (Gabriel et al., Chem Soc Rev 27:213–223, 1998) and another by Thostenson and Chou (Manufacturing 30:1055–1071, 1999), summarizing the fundamentals and several applications of microwave processing of chemical compounds and composite materials, respectively. These review articles also present a direct comparison of microwave heating with conventional heating with respect to the processing of materials and microwave-assisted synthesis of chemical compounds. In this review article, we seek to remind the reader of the fundamentals of microwave heating and the interactions of microwaves with chemical and biological materials relevant to our recent work on bioassays, rather than repeating the information provided in the above-mentioned very informative reviews. We also summarize our work on MAMEF-based bioassays where we use plasmonic nanostructures to additionally plasmon-enhance fluorescence signatures.

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Keywords Metal-enhanced fluorescence · Plasmon-enhanced fluorescence · Surface-enhanced fluorescence · Plasmonics · Radiative decay engineering · Plasmonics · Electric field enhancement · Silver island films · Focused microwaves · Microwave-accelerated metal-enhanced fluorescence ultrafast kinetics · Immunoassays

Microwaves and conventional heating

Microwaves are electromagnetic waves with frequencies between 0.3 and 300 GHz. For the purpose of microwave heating, the two most commonly used frequencies are 0.915 and 2.45 GHz; the latter frequency is being used in domestic microwave ovens worldwide. In microwave heating, the electromagnetic energy interacts with the materials at the molecular level, where the electromagnetic energy is transferred and converted to heat through the motion of the molecules. This results in rapid and uniform heating of the materials throughout their volume (also referred as volumetric heating), especially when the size of the materials is smaller than the wavelength of the microwaves. In contrast, the conventional thermal heating of materials proceeds via conduction, convection, or radiation of heat from the surfaces of the material. More specifically, heat is transferred from the source to the material due to a thermal gradient via the three heat transfer mechanisms mentioned above and is dependent on several parameters: diffusion of heat and conductivity of materials. Conventional heating often requires much longer heating times as compared to microwave-based heating.

In addition to the most notable advantage of microwave heating over conventional heating, that is, the volumetric heating of the materials, microwave heating can also be utilized for selective heating of materials within a mixture or a composite. This arises from the differences in dielectric

properties of the materials, in a mixture or a composite, microwaves selectively couple to the nonmetallic material with a higher dielectric loss factor (ε″). The selective heating is the crux of the microwave-accelerated metal-enhanced fluorescence (MAMEF) bioassay platform technology developed by our laboratories, where the assay medium is selectively heated to a higher temperature than the metallic nanoparticles: the thermal gradient rapidly driving the biorecognition events to completion due to temperature-induced mass transport of biomolecules from the warm assay medium to the colder assay medium. We offer a short summary of the interactions of microwaves with metals and chemical and biological compounds in the following sections. The reader is also referred to the review article by Thostenson [3] for the description of dielectric properties and for a summary of electromagnetic theory.

Microwaves and metals

Electronic conduction plays an important role in the microwave heating of metals due to their large conductivity values. In conductors, electrons move freely in the material in response to the electric field and result in an electric current flowing through the metal (Foucault currents). Unless it is a superconductor, the flow of electrons will heat the material through resistive heating. However, microwaves will be largely reflected from metallic conductors, and therefore, they are not effectively heated by microwaves. For metals with large conductivity values, the electric and magnetic fields attenuate rapidly toward the interior of the metal due to the so-called "skin effect." When a large current flows inside the metal with high conductivity, a combination of the magnetic field with the current produces a force that pushes the conducting electrons towards an area adjacent to the boundary (skin depth), concentrating the electric fields. At 2.45 GHz, the skin depth for gold and silver nanoparticles lies within the micrometer range [4]. When the skin depth is larger than the dimensions of the metal, the "skin effect" is negligible. Thus, for metallic nanoparticles, the microwave heating is uniform throughout the dimensions of the nanoparticles. It was previously calculated that the temperature increase around the gold nanoparticles due to microwave heating (at 12 and 2.45 GHz) is in the order of micro-Kelvins [4, 5]. We have previously shown that, when the metallic nanoparticles placed in a aqueous medium are heated with microwaves for 20 s, the temperature of the aqueous medium increases by 5°C [5], creating a thermal gradient between the nanoparticles and the aqueous medium. The thermal gradient is the major driving force for the faster biorecognition kinetics observed near to metallic nanoparticles, i.e., an increased mass transfer to the plasmonic MEF surface.

Microwaves, water, and biological materials

Since the MAMEF-based bioassays are run in aqueous systems, the dielectric properties of water are clearly relevant to the efficiency of microwave heating of the assay components for faster bioassay kinetics. At 20°C, water has a relaxation frequency of 18 GHz or a relaxation time of 9 ps (loss factor is at maximum) [2], where the most efficient heating of water occurs. However, since the cost of microwave equipment to maintain the 18-GHz frequency for domestic microwave ovens is high, 2.45 GHz is usually used in these appliances. Nevertheless, water is still effectively heated at 2.45 GHz (loss factor for water at 2.45 GHz is one-fourth that of its value at 18 GHz) [2].

The other important component in a MAMEF-based bioassay is the biomolecules. Proteins/antibodies usually have a molecular weight several orders of magnitude greater than that of water; thus, the dielectric dispersion region for proteins occurs at lower frequencies than water [2]. It was shown that the dielectric dispersion region for a 68-kDa protein (hemoglobin) occurs at the 1–10 MHz range [2]. While for low protein concentrations the dielectric dispersion of water is unaffected by the presence of proteins, high protein concentrations (in the molar range) result in a dielectric dispersion, which occurs at frequencies typically an order of magnitude less than water. That is, an aqueous protein solution will be heated more effectively at a frequency around 2 GHz. It is also important to note the effect of ionic salts used along with most biomolecules on the dielectric properties of water. The presence of ionic salts (at concentrations used in buffer solutions, e.g., 1.8% KCl) increases the water relaxation time, enhancing the efficiency of microwave heating slightly [2].

MAMEF bioassay platform technology

MAMEF is a platform technology that couples the benefits of low-power microwave heating with metal-enhanced fluorescence (MEF), to address the two major shortcomings of fluorescence-based bioassays currently in use today; i.e., bioassay sensitivity and rapidity. In MAMEF [1], the MEF phenomenon increases the sensitivity of the assays, while the use of low-power microwave heating kinetically accelerates assays to completion within only a few seconds.

In MEF, usually, a low-quantum-yield fluorophore is placed near metallic nanostructures and two significant observations are made: significantly increased fluorescence emission and a reduced lifetime of fluorophores [6, 7]. The reduction in fluorescence lifetime ultimately results in fluorophores becoming more photostable near metallic nanoparticles. More specifically, the excited fluorophores partially transfer energy to the silver nanoparticles, as the emission

from the fluorophore–silver "system" radiates more efficiently than the emission from the fluorophores alone [8–10]. For a more detailed description of MEF, the reader is referred to numerous publications found in the literature [6, 8–16].

The three major components of the MAMEF bioassay platform technology are (1) silver nanoparticles, (2) biomolecules, and (3) aqueous medium. The silver nanoparticles serve as (1) a platform for the attachment of one of the biorecognition partners (anchor probe), (2) an enhancer of the fluorescence emission via surface plasmons [9], and (3) a mediator for the localized delivery of the microwave energy that significantly speeds up the biorecognition process due to localized heating. It is therefore prudent to study the effects of microwave heating on silver nanoparticles. It should be noted that we have focused on silver as the metal of choice, although our rationale applies to any plasmon resonance-supporting nanoparticle [17].

Figure 1 summarizes characterization studies for silver island films (SiFs) [1] before and after microwave heating. A typical photograph of SiFs-deposited glass substrate is shown in Fig. 1a, where SiFs are the substrate studied the most to date [6] for applications in MEF. The SiFs were deposited onto the right side of the glass substrate, and the left side is left intentionally blank for control studies. Figure 1b shows the plasmon absorption spectra of the SiFs, both before and after low-power microwave heating for 30 s. As can be seen from Fig. 1b, the microwave heating had no effect on the surface plasmon absorption of the SiFs, indicating no changes in structure or surface shape of the silver nanoparticles. Furthermore, no "sparking" was evident from the silvered surfaces, a known consequence of surface charge build-up and dissipation [1], for large-wavelength-sized particles or continuous surfaces. The structural morphology of the silvered surfaces was also measured using atomic force microscopy; Fig. 1c. Little to no change in surface morphology was observed, clearly demonstrating the compatibility of the nanostructured metallic surfaces to microwave heating.

The thermal gradient created between the bulk of the aqueous medium and the metallic nanoparticles during microwave heating is one of the major reasons for the observed faster biorecognition kinetics in MAMEF-based bioassays. Therefore, it has been informative to determine the temperature of the assay components during the microwave heating process. In this regard, two approaches have been employed: (1) "indirect" [1] and (2) "direct" methods [18]. In the former, the temperature changes during the microwave heating can be determined by monitoring the ratiometric absorbance response of a temperature-sensitive dye. Thymol blue is heated in the microwave cavity and is quickly transferred to the spectrophotometer, where the absorption spectrum is measured [1]. Figure 2a and b show the temperature-dependent absorption spectra of thymol blue and the assay

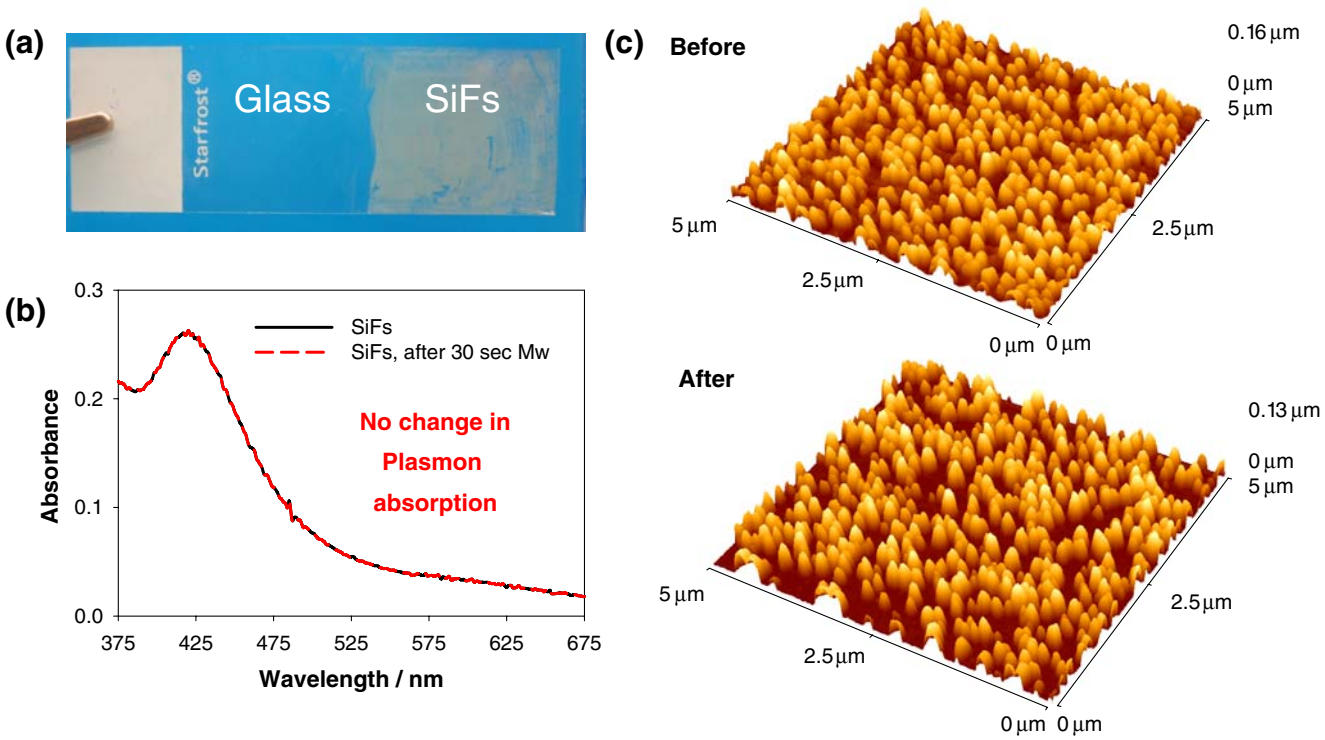

Fig. 1 Sample characterization studies. **a** A typical photograph of silver island films (*SiFs*) deposited glass substrate, **b** plasmon absorption spectra both before and after microwave heating, and **c** the corresponding AFM images. Adapted from [1]

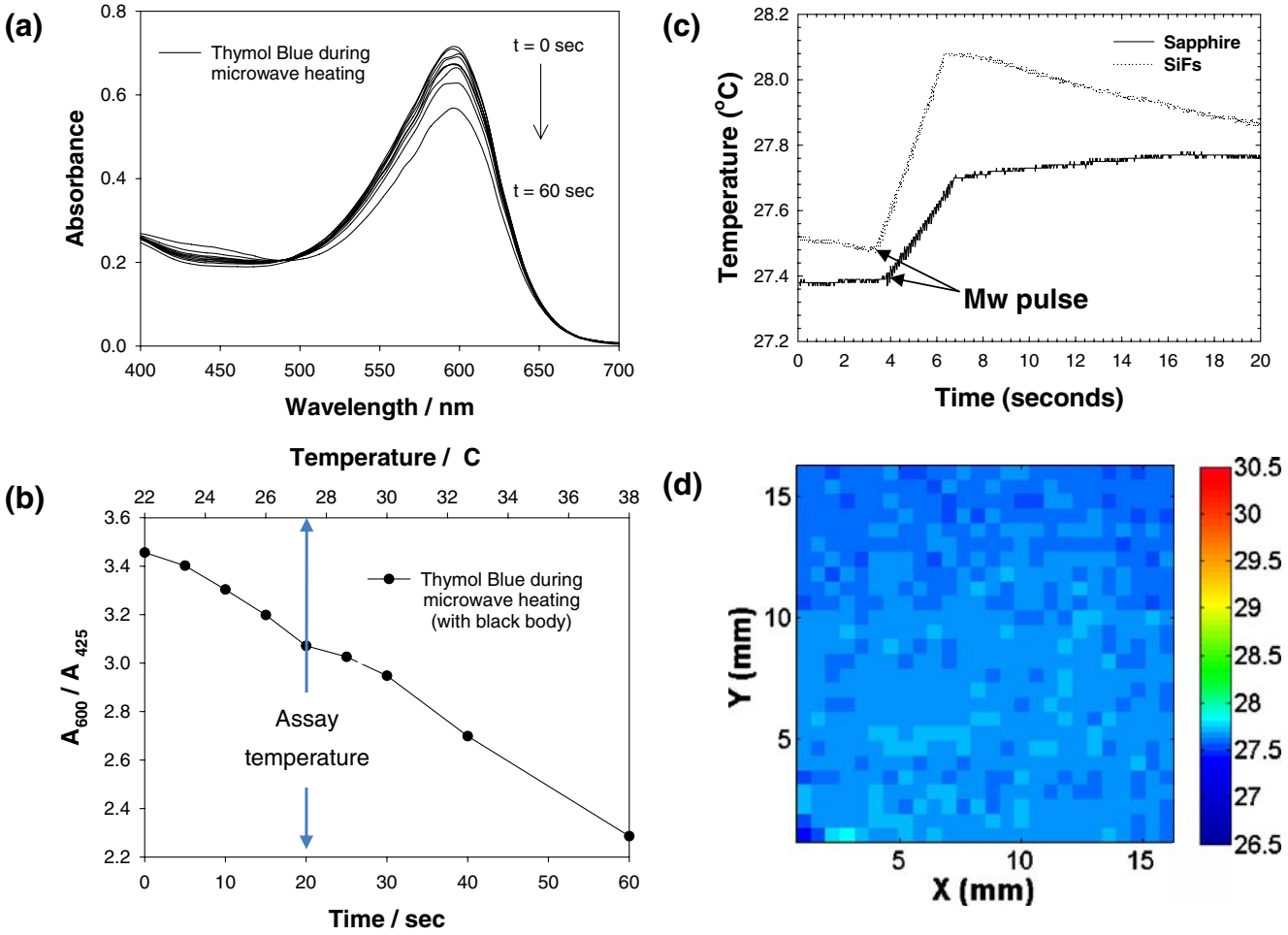

Fig. 2 Real-time monitoring of temperature changes during microwave heating. **a** Absorption spectra as a function of temperature for 30 μl thymol blue measured during microwave heating; **b** the respective absorbance, temperature vs time ratiometric plot; **c** real-time temperature distributions of water on a SiFs-deposited sapphire substrate captured using a thermal camera; **d** a thermal image of SiFs during microwave heating. Adapted from [1] (**a**, **b**) and [18] (**c**, **d**)

temperature calibration curve for microwave heating up to 60 s, respectively. From these calibration plots, a 20-s microwave exposure (140 W, 2.45 GHz) results in a temperature jump of ~5°C (to ≈28°C) for 30 μl of sample. Hence, with this calibration curve, one can simply change the assay surface temperature by changing the duration of the microwave heating. It is important to note that new calibration curves are needed for larger sample volumes.

In the "direct" method [18], the determination of temperature changes on the assay surface during microwave heating is undertaken using a thermal imaging camera that captures the infrared (IR) radiation, giving a high-speed and high-sensitivity determination of the actual temperature. In order to detect the IR radiation, glass substrates are replaced with sapphire plates that transmit IR radiation, the SiFs simply being deposited on the sapphire plate. Figure 2c and 2d show the results for real-time monitoring of the temperature during microwave heating. From mean temperature vs time plates for blank sapphire (no SiFs) and silvered sapphire sample geometries, a slightly higher thermal gradient is observed for the water on the silvered sapphire substrates (Fig. 2c). It is important to note that, after the microwave heating is turned off, while the temperature of water remains constant on the blank sapphire substrate, the water starts to cool down rapidly close to the silvered sapphire substrate. This implies that the heat is transferred from the warmer water (due to microwave heating) to the colder silver nanoparticles (which are not heated during microwave heating), inevitably resulting in the faster diffusion of the biomolecules towards the silver nanoparticles. A thermal image (Fig. 2d) shows that the temperature distribution is uniform on the silvered sapphire sample geometries 2 s after the onset of microwave heating. Interestingly, since the biomolecular binding events occur close to the cooler plasmonic nanostructures, the surface-bound biomolecules do not experience higher temperatures and are, in essence, protected from possible thermal denaturation.

MAMEF-based protein assays

The original proof-of-principle of MAMEF-based bioassays was first demonstrated with a model protein detection assay [1]. Figure 3a shows the model protein–fluorophore system that was coated equally on half of a silvered glass substrate, and the other half of the glass substrate was left intentionally blank to compare the benefits of the MEF phenomenon. In the model protein assay, one of the binding partners [biotinylated-bovine serum albumin (BSA)] is attached to the surface and is allowed to bind to its binding partner (fluorescein-labeled streptavidin) by incubation at room temperature or through low-power microwave heating. Figure 3b, left, shows that the fluorescein emission intensity from the silvered substrate is ≈6-fold greater than that from the glass substrate for the assay run at room temperature for 30 min. Figure 3b, right, shows the combined effect of both low-power microwave heating (20 s) and the MEF effect for the identical assay run at room temperature. The microwave-accelerated assay yielded a similar fluorescence intensity after just 20 s as compared to the assay run at room temperature for 30 min. The emission intensity from the silvered substrate is ninefold greater than that from the glass substrate for the microwave-accelerated assay. This observation is thought to be due to the reduced extent of binding on glass as compared to the silvered side where the thermal gradient is larger. That is, one would need longer microwave heating times on glass substrates to achieve >95% completion or the equivalence of the assay. Real-color color photographs (Fig. 3b, right, inset) taken through an emission filter provide visual evidence for the larger fluorescence emission intensity measured from the silvered substrates.

In addition, three different control experiments, as shown in Fig. 4, were also run to confirm the efficacy of the MAMEF assays. Figure 4a shows that no fluorescence emission intensity was detectable from the control assay, where biotinylated-BSA is omitted from the assay, which was run at room temperature for 30 min. When the identical control assay was run with microwave heating, once again there was little fluorescence emission. These two control experiments nicely demonstrated that the nonspecific binding of the fluorescein-labeled streptavidin to the surface was minimal. In the third control experiment, the importance of the assay time was assessed, where the full assay was allowed to run at room temperature for 30 s (corresponding to the microwave-accelerated assay time) and readily shows the assay is incomplete, as shown in Fig. 4c.

The fluorescence lifetime of a fluorophore is indicative of its environment and was proven to be a useful tool to show the benefits of MEF [7]. It was previously shown that, when

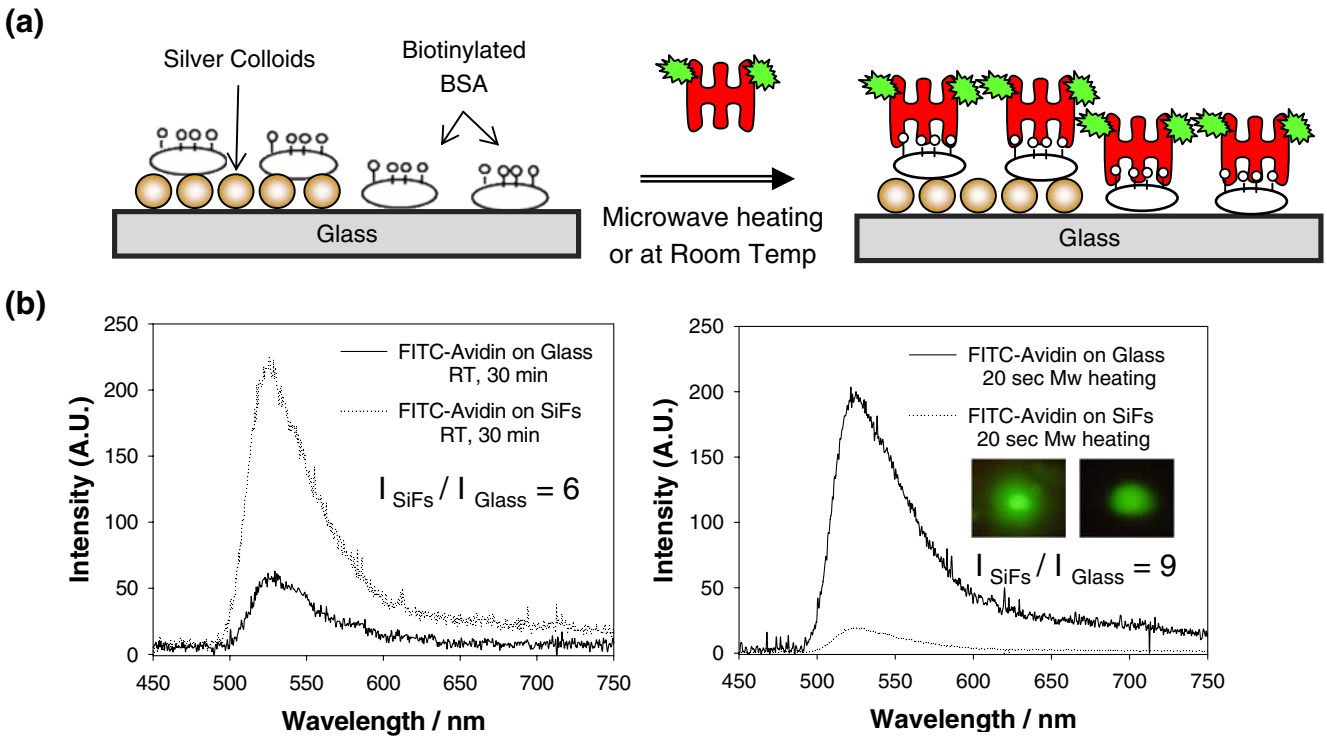

Fig. 3 A model MAMEF-based protein detection assay. **a** Model protein-fluorophore system used to demonstrate MAMEF on glass substrates; **b** emission spectra of flourescein isothiocyanate (*FITC*) for a model protein assay run at room temperature for 30 min and with microwave heating for 20 s. *RT*, room temperature. Adapted from [1]

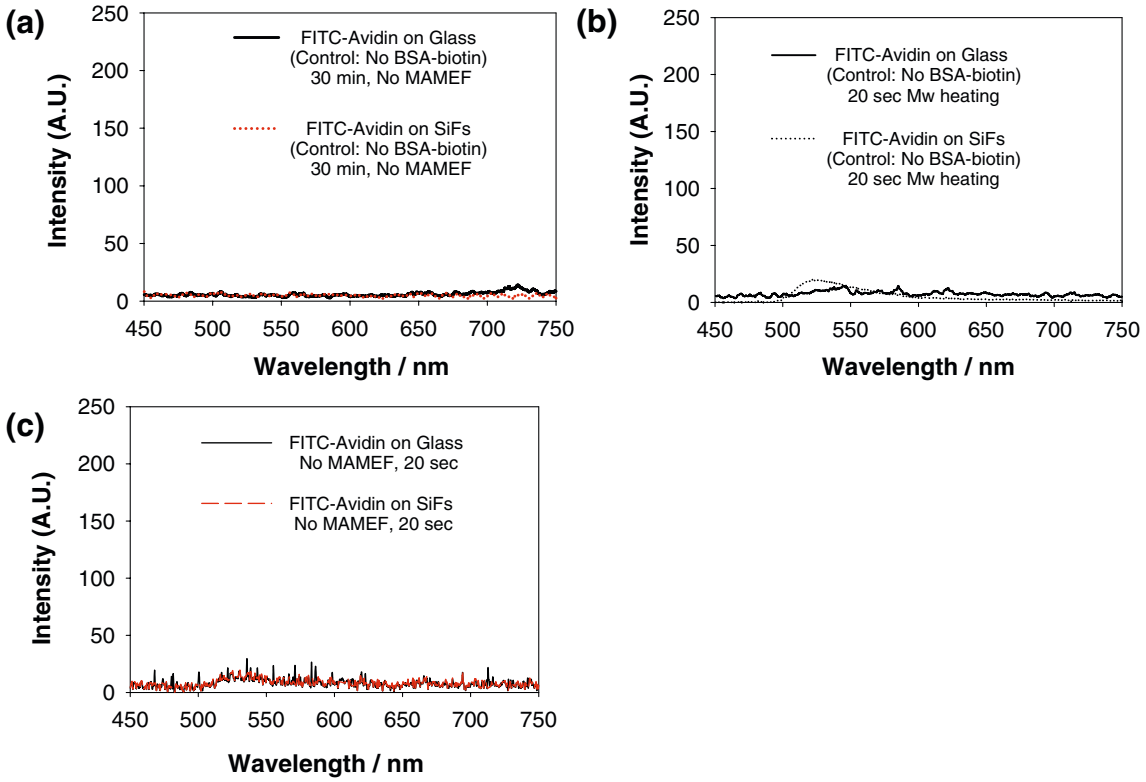

Fig. 4 Control experiments for the model MAMEF-based protein detection assay. **a** Control experiment, where one of the protein binding partners, BSA-biotin, is omitted from the assay, run at room temperature; **b** same control experiment with microwave heating for 20 s; **c** model protein detection assay run at room temperature for 30 s. *FITC*, flourescein isothiocyanate. Adapted from [1]

placed near metallic nanostructures, fluorophores have shorter lifetimes (as can be identified by multiexponential decay) as compared to free-space solution or on glass substrates. In an MAMEF-based assay, the lifetime information can be useful to assess the extent of completion of the assay: if the assay run at room temperature and with microwave heating goes to >95% completion (30 min and 20 s, respectively), then the lifetime of the fluorophores is expected to be very similar. Figure 5 shows that the fluorescence intensity decay curves for fluorescein after 30 min incubation and 20 s microwave heating were almost identical and significantly reduced as compared to the glass control. These results strongly indicate that the assays are virtually identical after 30 min incubation at room temperature and after 20 s microwave heating. Remarkably, it was also demonstrated that the assay does not undergo any protein conformational changes due to low-power microwave heating, as evidenced by resonance energy-transfer studies, where the donor–acceptor distance remained constant during microwave acceleration (i.e., heating) [1].

Fluorescence-based detection is also the basis of many assays in high-throughput screening (HTS) in use today. The sensitivity and rapidity of fluorescence-based HTS applications are underpinned by the quantum yield of the labeling fluorophore and the kinetics of the antigen–antibody recognition step [19–21]. Based on the success of our model MAMEF assay on silvered glass substrates [1], our group has also shown the application of the MAMEF

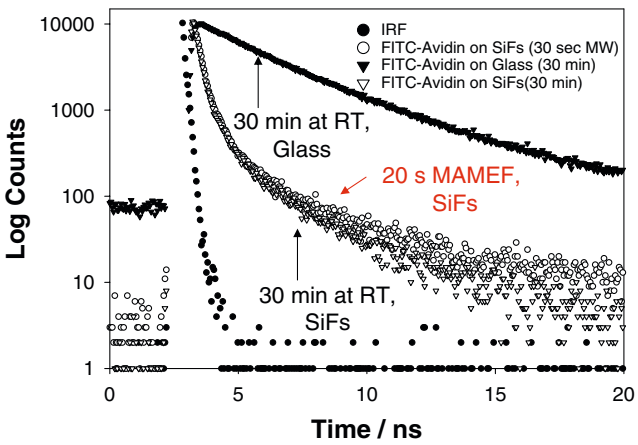

Fig. 5 Fluorescence lifetime studies for the fluorophore used in the MAMEF-based protein detection assay. Intensity decays for flourescein isothiocyanate (*FITC*)-Avidin on both glass and SiFs, both before and after exposure to low power microwave heating. The intensity decays on the SiFs are almost identical. *IRF*, instrument response function. Adapted from [1]

technology to assays run in an HTS format [22]. In this regard, commercially available polylysine-coated HTS wells were modified with silver nanoparticles (Fig. 6a) and a model protein assay run at room temperature and with microwave heating. Figure 6b shows the fluorescence emission intensity from both silvered and nonsilvered HTS wells after room temperature incubation (for 30 min) and after microwave heating (30 s). The fluorescence emission intensity is ≈5- and 4-fold greater from silvered wells as compared to nonsilvered wells, for assays run at room temperature and after microwave heating, respectively. This study [22] shows, in principle, the applicability of the MAMEF technology to high-throughput fluorescence-based applications, such as drug discovery, biology, and general compound library screening.

MAMEF-based immunoassays

In the previous section, we summarized the proof-of-principle demonstration of the MAMEF technology with a model two-component protein-detection assay. Most commercial protein detection systems in use today are based on the specific recognition of bivalent antigens with antibodies in several immunoassay formats [20]. Once again, fluorescence is the dominant detection technology in immunoassays due to the availability of fluorophores over a wide range of wavelengths and quantum yields. The immunoassays usually take anywhere from 10 min (with an expensive commercial unit) up to a few hours (in HTS format), which involves numerous incubation and washing steps [20].

Recently, we demonstrated the applicability of the MAMEF technology to a cardiac marker immunoassay to significantly reduce the assay time and sensitivity [23]. Figure 7a shows the experimental details of the myoglobin immunoassay that was constructed on silver-deposited glass microscope slides. In this regard, a capture anti-myoglobin antibody is adsorbed onto silver nanoparticles after an overnight incubation. The subsequent myoglobin and Alexa 647-labeled anti-myoglobin antibody binding steps were carried out either at room temperature or with low-power microwave heating; each step included either 30 min of incubation at room temperature or a 20-s microwave heating and a washing step to remove the unbound myoglobin. Figure 7b shows fluorescence emission spectra of Alexa-647 measured after the final binding step was carried out with low-power microwave heating and at room temperature (in separate experiments) on silver and on glass (a control sample to show the benefits of MEF). After a 30-min incubation at room temperature, the fluorescence emission intensity of Alexa 647 from the silvered side was ≈7.5-fold larger than the intensity from the glass control, showing one the benefits of MEF (increased fluorescence emission); Fig. 7b, left. When the identical immunoassay was run with low-power microwave heating (Fig. 7b, right), a similar final emission intensity was observed as compared to the assay run at room temperature (Fig. 7b, left). These results clearly demonstrate the

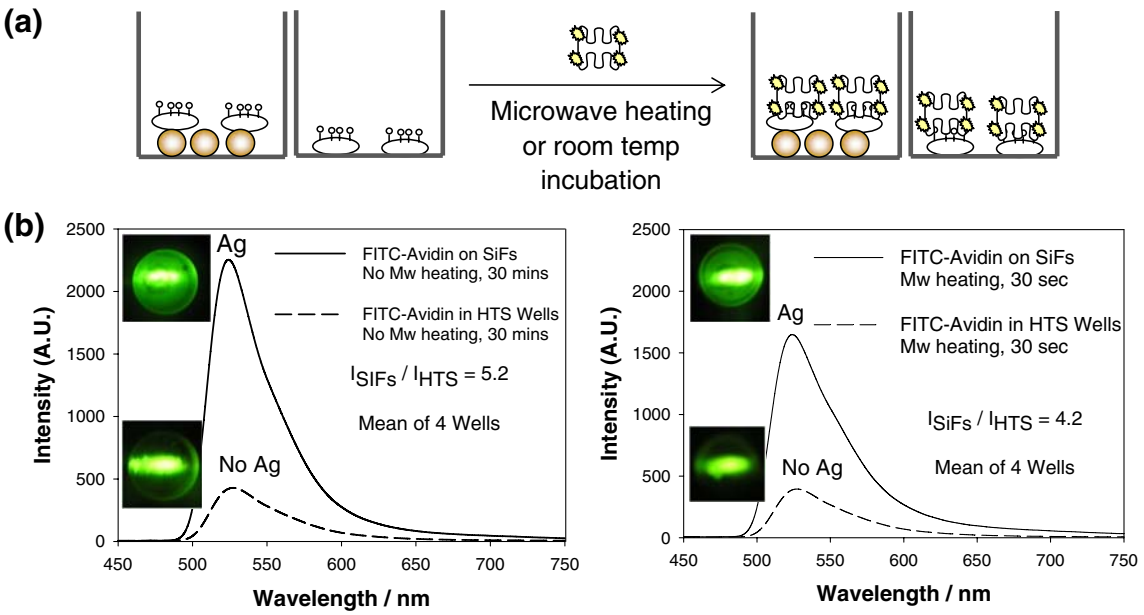

Fig. 6 A model MAMEF-based protein detection assay for HTS wells. **a** Model protein-fluorophore system used to demonstrate MAMEF in HTS wells; **b** emission spectra of FITC for a model protein assay run at room temperature for 30 min and with microwave heating for 20 s. *Room temp*, room temperature. Adapted from [22]

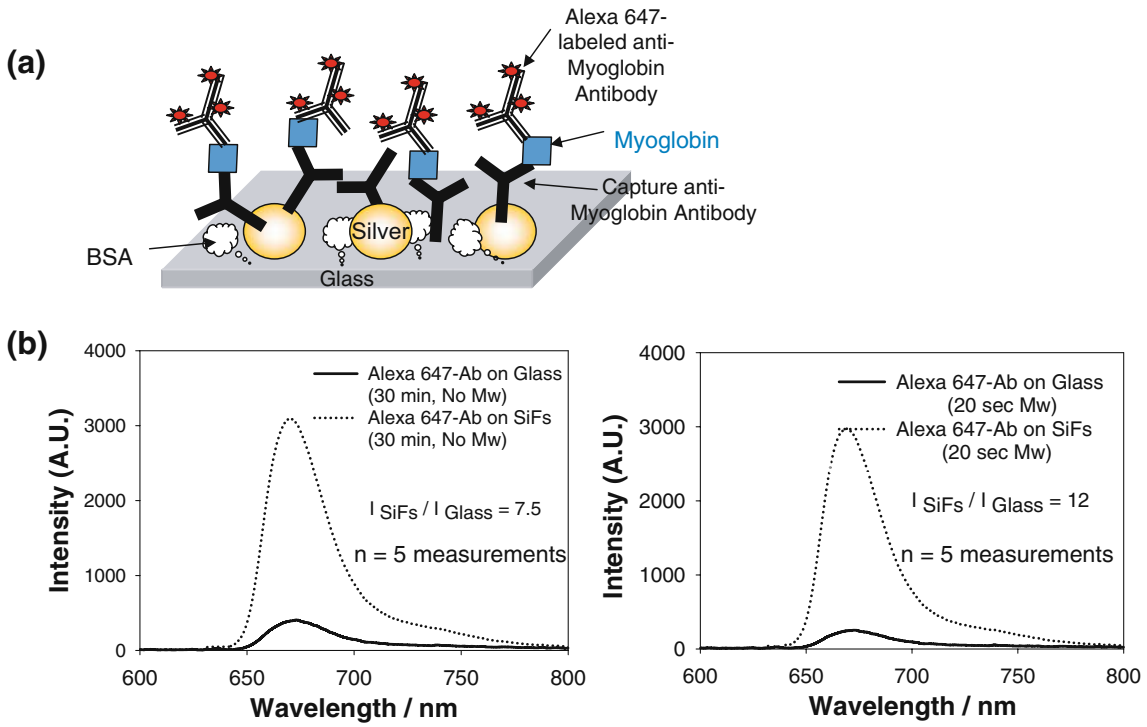

Fig. 7 MAMEF-based myoglobin detection assay on glass substrates. **a** Myoglobin immunoassay; **b** fluorescence emission spectra of Alexa-647 for the myoglobin immunoassay on SiFs-deposited glass substrates after low-power microwave heating and with no microwave heating. These results were obtained on silvered glass microscope slides. Adapted from [23]

applicability of MAMEF technology to a myoglobin immunoassay and, potentially, AMI screening.

It is interesting to note that, after 20 s of microwave heating, the emission intensity of Alexa-647 from the silvered side was 12-fold larger than the intensity from glass control. This is thought to be due to the combination of localized heating around the silver nanoparticles and the thermal gradient that is created between the bulk of the water and the silver nanoparticles, which results in the mass transport of biomolecules from the solution to the silver surface within 20 s. In control experiments (data not shown here) [23], where myoglobin is omitted from the assay, the emission intensity of Alexa 647 from both the silvered side and the glass control was negligible.

MAMEF-based DNA hybridization assays

DNA hybridization assays are routinely used in many diagnostics applications [24], on gene-chips [25] and fluorescence in situ hybridization [26]. In all of these applications, usually fluorophores with high-quantum yield are employed to increase the sensitivity of the hybridization assays, raising several issues such as high background emission and photostability of the fluorophores. Ideally, to maximize the efficiency and, when required, the sensitivity of the DNA hybridization assays, it would be beneficial to employ low-quantum-yield fluorophores, which can withstand the long exposure to excitation light [6]. The use of plasmonic nanoparticles with low-quantum-yield fluorophores (MEF) increases the sensitivity of the DNA hybridization assays, as well as the photostability of the fluorophores [6]. However, one has little or no control over the rapidity of the DNA hybridization assays. In this regard, we recently demonstrated the applicability of the MAMEF technology for rapid and sensitive DNA hybridization assays, where two complementary oligonucleotides (one labeled with a fluorophore) were hybridized on silver nanoparticles within 20 s, after low-power microwave heating (duty cycle of 6 s) with the hybridization believed to be over in <5 s [27, 28].

Figure 8a shows the experimental configurations of the DNA hybridization assay constructed on silver-deposited glass microscope slides. In this regard, a 23-mer anchor probe (capture oligonucleotide) was attached to silver nanoparticles via a sulfhydryl-metal bond after an overnight incubation. The DNA hybridization assay was carried out either by incubation of a fluorescein-labeled complementary oligonucleotide on silvered glass at room temperature for 3.5 h, or with low-power microwave heating for 20 s. Figure 8b shows the fluorescence emission spectra of fluorescein after 3.5 h room temperature incubation and after 20 s of microwave heating. Control experiments, where the anchor probe is omitted from the surface, corresponding to the DNA hybridization assays are also

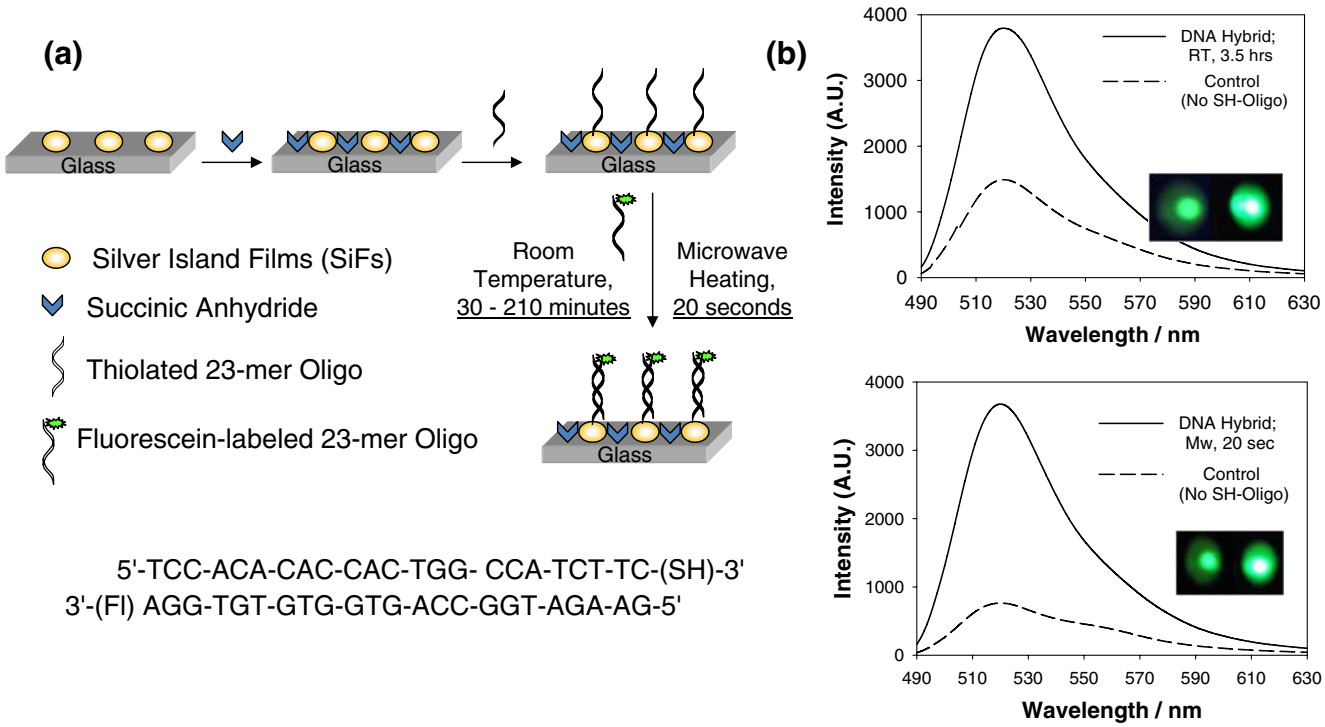

Fig. 8 A model MAMEF-based DNA hybridization assay on silvered glass substrates. **a** Model DNA hybridization assay; **b** emission spectra of fluorescein-oligo (3 mM) after MAMEF-based and room temperature hybridization on SiFs. *Insert photographs*, both before and after hybridization is completed. Adapted from [28]

shown in Fig. 8b. After 3.5 h incubation at room temperature, fluorescein emission intensity from the hybridization assay is ≈2.5-fold larger than the intensity from the corresponding control assay, which is also evident from the real-color photographs taken through an emission filter. Identical fluorescein emission intensity is observed after 20 s (a >600-fold decrease in assay run time) from the MAMEF-based DNA hybridization assay.

Nonspecific binding is also a major factor that influences the sensitivity of the DNA hybridization assays. In MEF-based assays, silver nanoparticles serve two purposes: (1) enhancement of close-proximity fluorescence emission and increased fluorophore photostability of fluorophores and (2) as a platform to attach the anchor oligonucleotides. The latter requires that silver nanoparticles are protected against nonspecific binding of complementary target oligonucleotide. In this, regard, self-assembled monolayers of a short-chain (up to 10 methylene) alkanethiols chemically nonreactive terminal groups can be used to occupy the spaces between the anchor probes on the surface of plasmonic nanoparticles [27, 29].

When employed in MAMEF-based DNA hybridization assays, silver nanoparticles also localize the heat around them, which contributes to the faster hybridization kinetics on the surface. The faster hybridization kinetics has implications on the extent of the nonspecific binding of complementary target oligonucleotides to silver. Since the DNA hybridization is completed within 20 s after microwave heating, a much lesser extent of nonspecific binding is typically observed in MAMEF-based hybridization assays as compared to hybridization assays carried out at room temperature [27]. It is important to note that a reduction in nonspecific binding results in better sensitivity in the DNA hybridization assays and is thought to be due to a change in k_D during microwave heating.

One of the most important factors in MAMEF-based DNA hybridization assays is the effect of microwave heating on the oligonucleotides themselves. Our research group has previously shown that proteins do not denature when exposed to low-power microwave heating, which was demonstrated using fluorescence-resonance energy transfer studies [1]. In an analogous manner, the effects of low-power microwave heating on the ability of DNA to both melt and rehybridize with additional complementary target oligonucleotide was also studied [27]. Figure 9 shows the emission spectra of fluorescein-labeled oligonucleotide (a) after 20 s of microwave heating (final step of the MAMEF-based DNA hybridization assay), (b) after melting the DNA and removing the fluorescein-labeled oligonucleotide using warm buffer above the melting point of the ds-DNA, and (c) after rehybridization with fresh fluorescein-labeled oligonucleotide

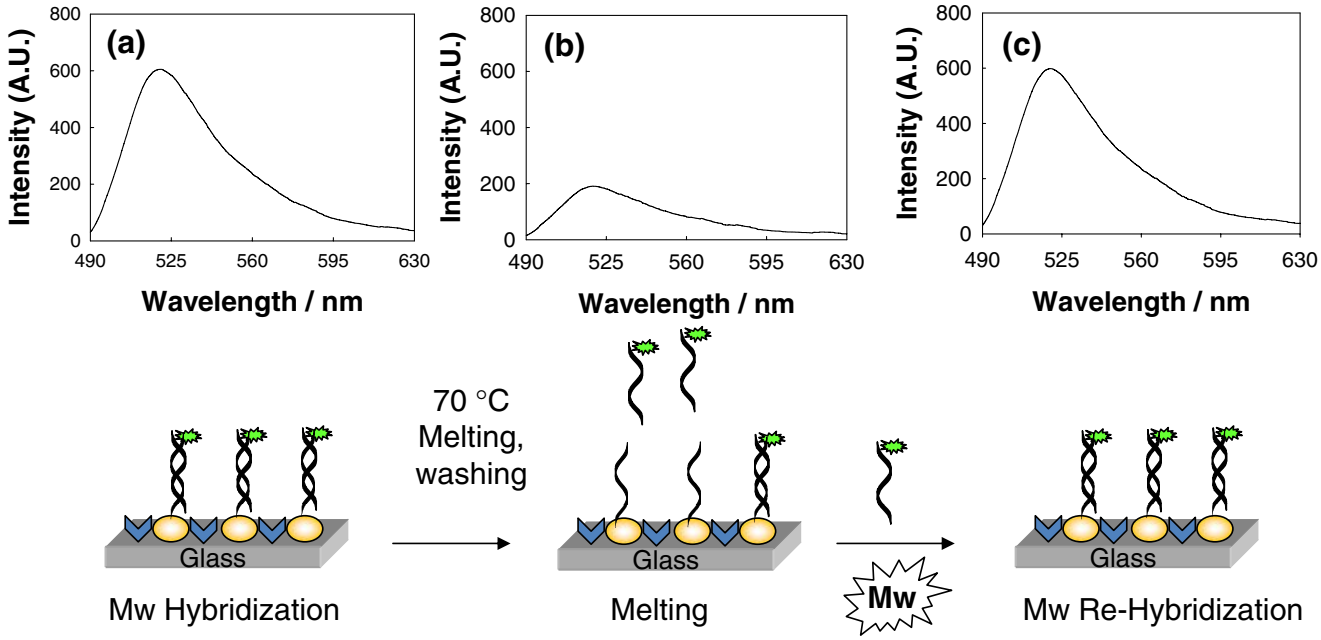

Fig. 9 Reversibility of the model DNA hybridization assay on glass substrates. Emission spectra of fluorescein-DNA **a** after hybridization, **b** after melting at 70°C, and **c** after a further 20-s low-power microwave heating with an additional 250 nM Fl-DNA. The silvered surface was washed with buffer several times between each measurement. Adapted from [28]

using microwave heating. As one can see, after the rehybridization is completed with microwave heating (Fig. 9c), a similar fluorescein intensity is observed, indicating that the anchor probe on the silver surface is unaffected during microwave heating. These results imply that silvered surfaces with anchor oligonucleotides are reusable, an important factor in the preparation of low-cost MAMEF-based DNA hybridization assays.

MAMEF-based anthrax detection

In the previous section, we summarized the application of the MAMEF technology to DNA hybridization assays using a two-piece oligonucleotide model hybridization assay [27], where the target oligonucleotide is labeled with a fluorescent probe. However, in a "real-world setting" it would not be practical to label the target oligonucleotide for the detection of target DNA. A common practice is to employ a third oligonucleotide labeled with a fluorophore (fluorescent probe), a specific sequence that hybridizes with the target oligonucleotide at another location close to the anchor probe, i.e., a three-piece DNA hybridization assay. In this regard, we recently presented the applicability of MAMEF technology to the detection of target DNA encoding a region of the protective antigen gene of *Bacillus anthracis* (i.e., the disease anthrax) [30].

Figure 10a shows the experimental details of the target anthrax DNA assay constructed on silver-deposited glass microscope slides. In this DNA detection scheme, the anchor probe is attached to silver nanoparticles through sulfhydryl groups. In order to minimize the nonspecific binding of DNA to the assay platform, silver nanoparticles and glass are modified with additional surface-protective chemicals [30]. The subsequent hybridization of the fluorescent probe and target anthrax DNA (synthetic) was carried out in a single microwave heating step, followed by an orange emission at 585 nm through an emission filter when excited with a green laser (532 nm). The intensity of the emission at 585 nm was directly related to the concentration of the target anthrax DNA, Fig. 10b, c, as was also evident from the real-color photographs of the emission taken through an emission filter. Control experiments, where the anchor probe is omitted from the assay surface, showed emission intensities far less than those from the actual assay over the range of concentrations studied [30].

In addition to the detection of synthetic anthrax target DNA, our research group has recently shown the translation of the MAMEF technology to genomic DNA that was extracted from a vegetative culture, the Sterne strain of *B. anthracis*, using commercially available DNA extraction procedures (Promega, Madison, WI, USA). It is known that the exosporium (a loose-fitting, balloon-like layer surrounding the spore) of anthrax carries the genomic information derived from the mother vegetative cells [31], which can also be used in a DNA detection scheme. Figure 11 shows the orange emission measured from the MAMEF assay

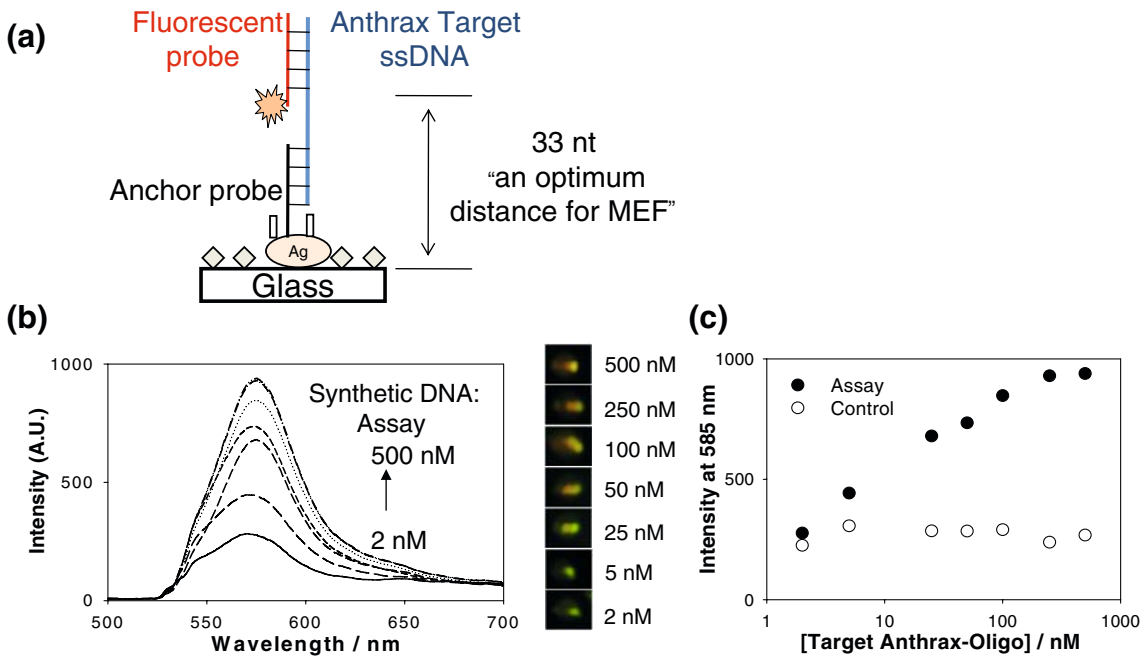

Fig. 10 MAMEF-based anthrax DNA hybridization assay on silvered glass substrates. **a** Experimental design, depicting the organization of the DNA oligomers on SiFs used for the detection of *B. anthracis*. **b** Emission spectra of TAMRA-ssDNA as a function of concentration after 30 s of low-power microwave heating. Photographs showing the increased emission intensity visually as a function of TAMRA-ssDNA concentration after 30 s of low-power Mw heating. **c** Semilogarithmic plot of the fluorescence emission intensity at 585 nm for the TAMRA-ssDNA as a function of the target synthetic DNA concentration. Adapted from [30]

after the exosporium sample containing target anthrax DNA was hybridized on the assay surface. Similar to data collected from the MAMEF assays for the detection of synthetic and genomic target anthrax DNA, the emission intensity at 585 nm was directly related to the concentration of exosporium, which was in the nanogram-per-microliter range. Control experiments, where the anchor probe was omitted from the assay surface, showed that the nonspecific binding of target DNA was significantly less than the lowest concentration of exosporium sample detected in the actual assay [30]. The ability of the MAMEF technology to distinguish between *Bacillus cereus*, a close relative of *B. anthracis*, and anthrax was also demonstrated [30]. Fluorescence emission intensity at 585 nm from this control assay shows constant emission intensity over a wide range of concentrations, indicating that the MAMEF assay platform clearly can distinguish between the two closely genetically related strains.

Summary and future outlook

Fluorescence has become one of the most widely used detection techniques in medical diagnostics and biotechnolo-

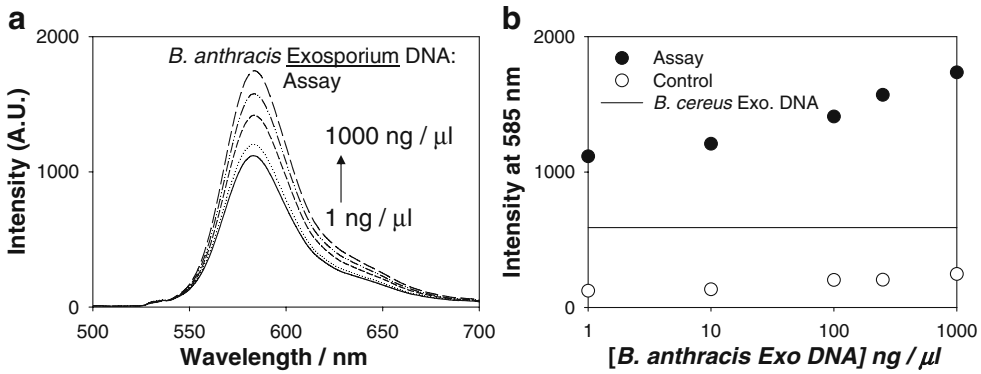

Fig. 11 MAMEF-based anthrax DNA hybridization assay on Silvered glass substrates. **a** Emission spectra of the TAMRA-Oligo as a function of *B. anthracis* exosporium concentration after 30 s of low-power microwave heating. **b** Plot of the fluorescence emission intensity at 585 nm for TAMRA-Oligo as a function of target concentration. Data for *B. cereus* (a noncausative strain) is also shown for comparison. Adapted from [30]

gy today. However, two main factors affect the efficacy of fluorescence-based detection: (1) high background sample/biological emission and (2) the photostability of the fluorophores. While the former results in the reduction of the sensitivity of fluorescence-based diagnostic tests, the latter limits the time for detection, especially for applications that required high irradiances or long detection times. Several approaches have been suggested to minimize the background signal caused by the sample medium, including fluorescence/polarization kinetic detection, time-gated detection based on long-lived lanthanide emission, and two-photon excitation. MEF, a phenomenon that results in the enhancement of fluorescence emission and an increase in the photostability of the fluorophores, allows scientists to readily address the issues of assay sensitivity and photostability.

Most biological recognition events, such as an antigen–antibody interaction or DNA hybridization, are often kinetically slow, requiring long incubation times. As a result, very few immunoassays can be completed in less than 10 min. In this regard, the MAMEF technology, which couples the benefits of MEF with low-power microwaves to accelerate bioaffinity reactions, offers the much needed solution.

In this timely review, we have summarized the fundamentals of microwave heating relevant to our recent work on bioassays. As we have shown here, the MAMEF technology can be applied to many fluorescence-based medical diagnostic tests, and in our opinion, the low cost and the simplicity of the MAMEF technology will allow the development of new point-of-care devices for the detection of a variety of clinical analytes and bioterrorism agents based on low-power microwave heating.

Acknowledgments The authors acknowledge the Middle Atlantic Regional Center of Excellence for Biodefense and Emerging Infectious Diseases Research (NIH NIAID—U54 AI057168) and National Institute of Neurological Disorders and Stroke NINDS—NS055187 and NS055187-S1. Salary support to the authors from UMBI and the IoF is also acknowledged.

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