Microwave-accelerated metal-enhanced fluorescence: an ultra-fast and sensitive DNA sensing platform

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In this paper, we investigated the effects of low-power microwave heating on the components of the recently described new approach to surface DNA hybridization assays, based on the Microwave-Accelerated Metal-Enhanced Fluorescence (MAMEF) platform technology. Thiolated oligonucleotides have been linked to surface-bound silver nanostructures which partially coat a glass slide. The addition of a complementary fluorescein-labeled oligonucleotide results in metal-enhanced fluorescein emission as the probe is brought into close proximity to the silver upon hybridization. In addition, the combined use with low-power microwave heating, which is thought to locally heat around the silvered surface, affords for both the assay kinetics and optical amplification to also be localized to the surface. In our model DNA target assay reported here, we can detect 23-mer targets in less than 20 s, up to a 600-fold decrease in the assay run time as compared to control samples hybridized to completion at room temperature. Importantly, the use of MAMEF also reduces the extent of unwanted non-specific DNA absorption, further increasing specific DNA target detection limits. It was also found that low-power microwave heating did not denature DNA and the bulk temperature increase near to silver nanoparticles was only *ca.* 1 °C.

Introduction

DNA capture assays are the basis of a wide range of biotechnology and diagnostics applications[1] and are routinely used in gene chips,[2] during PCR[3–5] and fluorescence-based *in situ* hybridization.[5] One can also find many examples of emerging DNA detection technologies based on aggregation of gold colloids (colorimetric),[6] and Surface Plasmon Resonance (SPR),[7–10] where the hybridization events are followed by the change in the reflectivity of the metal film and fluorophore–metal surface interactions,[11,12] and where the fluorescence emission from fluorophore-tagged oligonucleotides is monitored as a function of DNA hybridization. In most cases, the DNA capture assays are required to be highly sensitive, specific and fast whenever possible. The sensitivity of the fluorescence-based DNA hybridization assays is affected by multiple factors, which include unwanted background emission and the photostability of the fluorescent probe used. Moreover, DNA capture assays are often kinetically slow, requiring long incubation times. Therefore, there is an unequivocal need for the development of more sensitive and faster detection methods for more efficient DNA capture assays.

Metal-Enhanced Fluorescence (MEF), a technology that has been extensively studied over the last five years,[13,14] allows scientists to address the issue of assay sensitivity. In MEF, the fluorescence emission from a fluorophore positioned near to silver nanostructures will be significantly increased: a greater fluorescence emission is observed from fluorophores adsorbed on a silver surface as compared to glass alone (a control sample). Specifically, the excited fluorophores partially transfer energy (couple) to the silver nanoparticles, where the energy is rapidly and efficiently radiated, resulting in amplified emission from the fluorophore–silver 'system'.[15] MEF also results in a reduced fluorophore lifetime, the fluorophores becoming more photostable due to spending less time in an electronically excited state.[16] It is this unique combination of an increased emission intensity, coupled with a reduced lifetime, that has recently led to the use of MEF in many biotechnological applications, such as in the increased detectability and photostability of fluorophores,[13] and improved DNA detection to name just a few.

Recently, a new platform technology, Microwave-Accelerated Metal-Enhanced Fluorescence (MAMEF), which couples the benefits of MEF with low-power microwave heating to accelerate bioaffinity reactions, was recently introduced by the Geddes laboratories for bioassays,[17] DNA hybridization[18] and immunoassays.[19] In the MAMEF technology, the MEF phenomenon increases the sensitivity of an assay while low-power microwave heating kinetically accelerates assays to completion within a seconds.[17–20] The latter has been explained by the localized heating close to surface metal particles by microwaves. This localized heat dissipation is then used to reduce the activation energy of biorecognition events and to increase the diffusivity of the biomolecules above the metal particles. Recent fluorescence lifetime and fluorescence resonance energy transfer (FRET) studies have shown that microwave heating does not induce protein denaturation or environmental changes.[17] Therefore, MAMEF can provide

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for the realization of ultra-fast and ultra-bright DNA capture assays.

In this paper, a detailed study of the effect of low-power microwave heating on the components of the MAMEF assay platform for ultra-fast and sensitive DNA capture assays is presented, which significantly builds upon our initial rapid communication.[18] By combining the effects of silver nanoparticles on fluorophores with low-power, localized microwave heating, a model DNA hybridization assay was kinetically complete within 20 s. The hybridization kinetics were accelerated up to 600-fold, as compared to an identical hybridization assay run at room temperature. It was also found that the use of low-power microwave heating *significantly reduced* the non-specific binding of fluorescent-labeled target oligonucleotides providing a huge opportunity for more sensitive DNA hybridization assays to be realized. The microwave-induced temperature jump within close proximity to the silver nanoparticles was determined to be *ca.* 1 °C from studies using a thermally responsive fluorophore. Moreover, our studies show that DNA capture 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, strongly indicating no DNA denaturation.

Materials and methods

Materials

Silver nitrate (99.9%), sodium hydroxide (99.996%), ammonium hydroxide (30%), succinic anhydride, borate buffer, 1-methyl-2-pyrrolidone, D-glucose and premium quality APS-coated glass slides (75 × 25 mm) were obtained from Sigma-Aldrich. Oligonucleotides used in this work were obtained from the Biopolymer Core Facility at the University of Maryland School of Medicine.

Methods

Formation of silver island films (SiFs) on APS-coated glass slides. The deposition of silver island films onto glass slides was performed as described previously.[17]

Preparation of DNA capture assay silvered substrates. The preparation of silvered substrates for DNA capture assay was undertaken using a procedure that was published previously.[18] In this regard, a 500 μl aliquot of thiolated oligomer (500 nM), [5′-TCC-ACA-CAC-CAC-TGG-CCA-TCT-TC-(SH)-3′] was incubated overnight at 4 °C on the surface of SiF-deposited glass slides in Hepes buffer (5 mM Hepes, pH 7.5, with final concentrations of 100 mM KCl, 0.25 mM EDTA) followed by rinsing with water to remove the unbound material. To prevent both non-specific absorption and binding of oligomers onto the non-silvered regions, all free amino groups were subsequently blocked (unless stated otherwise) by incubation in freshly prepared succinic anhydride solution (0.156 M in 1-methyl-2-pyrrolidone, 20 mM borate buffer pH 8.0) for 15 min at room temperature, followed by rinsing with deionized water.

Microwave-accelerated and room temperature DNA capture assays. MAMEF-based DNA capture assays were performed by the incubation of 50 μl of fluorescein-oligo [3′-(Fl)-AGG-TGT-GTG-GTG-ACC-GGT-AGA-AG-5′] with a strand complementary to the thiolated oligomer immobilized on SiFs, in Hepes buffer for 20 s in a microwave cavity (a 0.7 cu ft, GE Compact Microwave Model: JES735BF, max. power 700 W). Room temperature DNA capture assay was performed using the procedure above, except that the assay was completed at room temperature instead of with microwave heating. In order to determine the extent of non-specific binding/adsorption of the fluorescein-oligo, the fluorescein-oligo was incubated on a succinic anhydride-blocked SiF surface, in the absence of immobilized thiolated oligomer, at room temperature for up to 210 min; or 20 s in the microwave cavity. The power setting of the microwave cavity was set to 2 which corresponded to *ca.* 140 W over the entire cavity.

Kinetics of the DNA capture assay. The hybridization kinetics of the fluorescein-oligo, annealed with the thiolated oligomer immobilized on the SiFs, were studied at room temperature using total-internal reflection evanescent wave excitation.[21]

Melting of ds-DNA and re-hybridization with microwaves. The melting of the ds-DNA (melting temperature ds-DNA: 59 °C) was accomplished by incubating the ds-DNA in 40 ml of Hepes buffer at 70 °C for 20 min with the buffer being replaced every 20 s, effectively washing the assay at high temperature. The re-hybridization of 250 nM fluorescein-labeled oligomer with the thiolated oligomer coated on the SiFs was achieved using microwave heating for 20 s, as described above.

Fluorescence measurements and real-color photographs. All fluorescence measurements were performed by collecting the emission intensity through a long-pass filter perpendicular to the assay surface, using a Fiber Optic Spectrometer (HD2000) from Ocean Optics, Inc., after total-internal reflection evanescent wave excitation using a 473 nm diode laser, Fig. 1(right). The real-color photographs of fluorescein-labeled DNA on SiFs were taken with an Olympus Digital camera (C-740, 3.2 Mega Pixel, 10× Optical Zoom) through the same long-pass filter as was used to record the emission spectra.

Temperature calibration in the microwave cavity. In order to both determine and calibrate the temperature jump during low-power microwave heating, the temperature-sensitive fluorophore, Acridine Yellow (AY) was employed. First, the temperature-dependent emission spectrum of AY was recorded in bulk solution using a Varian fluorometer as the temperature was gradually increased from 20 to 70 °C. The temperature-dependent spectrum, which was previously published,[18] was used to construct the calibration curve, *i.e.* normalized intensity (intensity at any time divided by the initial intensity(*versus* temperature. Subsequently, the emission spectra of AY on SiFs were recorded using an evanescent wave excitation geometry [Fig. 1(right)]. For the temperature calibration, this enabled information, *localized* to silver

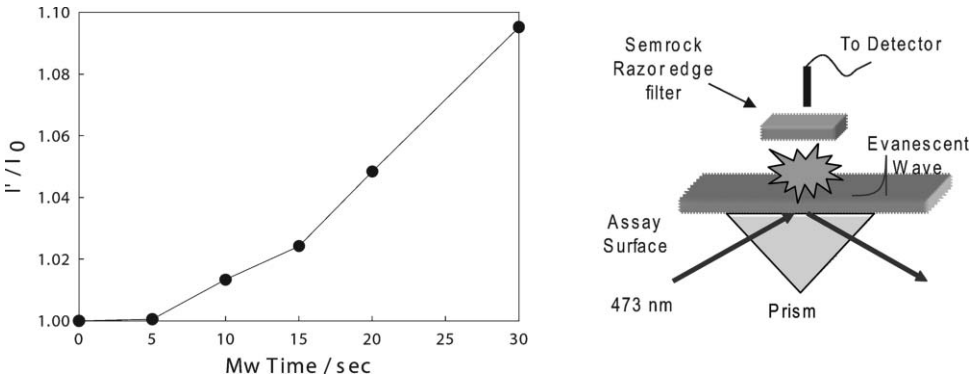

Fig. 1 (Left) Normalized emission intensity at 506 nm for Acridine Yellow sandwiched between two silvered slides *vs.* low-power microwave heating time measured using evanescent wave excitation. (Right) Experimental geometry showing the evanescent wave measurement.

nanoparticles, to be obtained. We note that the increase in *bulk temperature* due to microwave heating using front-face geometry was also previously published.[18]

Results and discussion

Surface DNA capture assays based on MAMEF and at room temperature were performed by the incubation of 50 μl of fluorescein-oligomer (fluorescein-oligo, Fig. 2) with a strand complementary to the thiolated oligo immobilized on SiFs for 20 s in a microwave cavity, or 30–210 min at room temperature. The extent of non-specific binding of fluorescein-oligo was determined by repeating the same experiment but in the absence of immobilized surface-thiolated oligo.

As discussed in the Introduction, MEF provides for enhanced luminescence signatures of fluorophores in close proximity to the silvered surface. Our assay shown in Fig. 2 was designed such that, after hybridization, the fluorescein labels on the target DNA's 3′-end will be 23 base pairs from the SiFs, approximately 7.5 nm. In all of our papers to date,[13,22,23] the maximum fluorescence enhancement (*i.e.* MEF) occurs for fluorophores positioned between 4 and 10 nm from the silvered surface. Subsequently, Fig. 3 shows real-time hybridization kinetics for both 5 and 500 nM target DNA concentrations, top and bottom, respectively. As the target DNA hybridizes and comes into close proximity to the silver, the signal intensity rapidly increases. Fig. 3 also shows

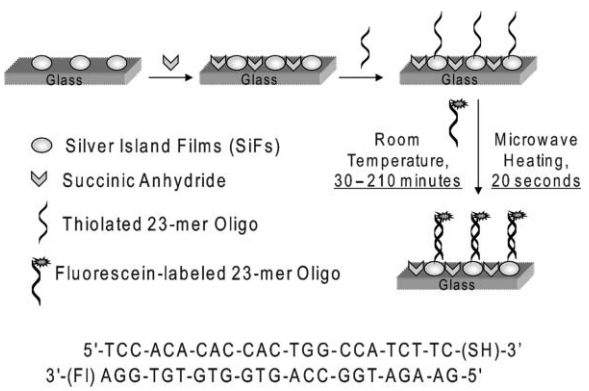

Fig. 2 MAMEF surface DNA capture assay. The lower panel shows the specific oligomer sequence used in this model study.

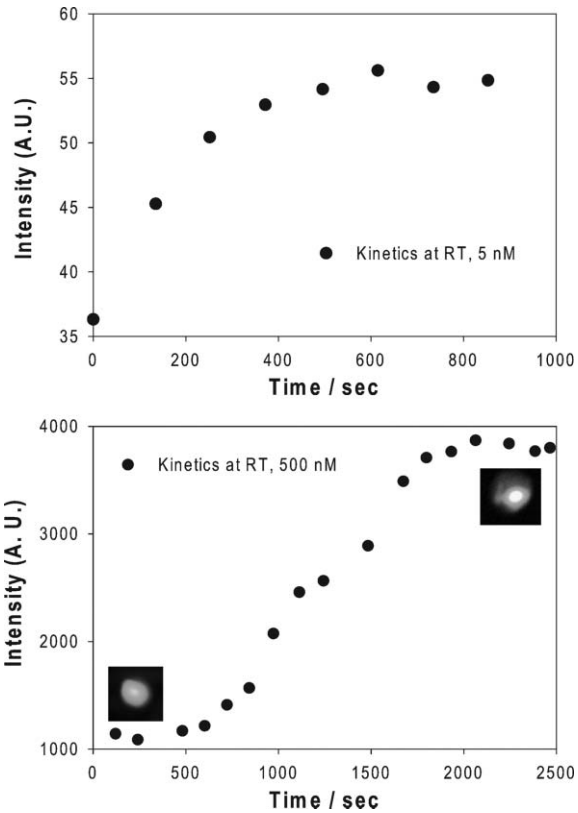

Fig. 3 Room temperature real-time hybridization kinetics for both 5 nM (top) and 500 nM (bottom) fluorescein-DNA (Fl-DNA). The photographs show the increase in fluorescein emission intensity after hybridization, a function of the close proximity of the fluorescein to the SiFs after hybridization. A.U. = arbitrary numbers.

this increase visually as seen through an emission filter [Fig. 3(bottom, inset)]. While the MEF phenomenon can provide for enhancement factors up to several thousand-fold as compared to a control sample containing no silver,[24] in our assay platform it is not feasible to develop an identical assay on a non-silvered substrate (control sample) to determine the true enhancement factor, *i.e.* the benefit of using silver, hence the <5-fold intensity change in Fig. 3 already accounts for significant silver enhancement in the first measurement. To this end, the evanescent wave mode of excitation employed

here allows for measurements close to the silvered surface to be achieved as compared to a traditional bulk fluorescence measurement.[21] While this mode of excitation is expected to sample only fluorophores up to 200 nm from the surface, this volume is still large in comparison with the 4–10 nm enhancement interaction volume close to silver colloids, accounting for the nearly 35 and 1000 A.U. of fluorescence intensity in Fig. 3(top and bottom), respectively.

The real-time hybridization kinetics shown in Fig. 3 clearly demonstrate that the initial intensity is higher, and longer hybridization times are required as the concentration of the fluorophore-labeled target oligo is increased. However, Fig. 3 does not provide information about the extent of non-specific binding of fluorescein-DNA. In this regard, to determine the contribution of non-specific binding to the detected signal, we have also measured the fluorescence emission spectrum of 3 μM fluorescein-DNA on SiFs with (hybridization) and without (control) thiolated oligo as a function of hybridization time at room temperature, Fig. 4. Fig. 4(top) shows an increase in emission intensity both on the hybridization and control assays as the hybridization time in changed from 30 to 210 min. The plot of emission intensities [from Fig. 4(top)] *versus* hybridization times reveals that the extent of non-specific binding is significantly higher for a high concentration of fluorescein-DNA.

In our previous publication,[18] we showed that by combining the effects of silver nanoparticles on fluorophores with low-power localized microwave heating, the hybridization kinetics of a model assay were accelerated significantly, as compared to an identical hybridization assay run at room temperature. However, we previously never investigated the effect of microwave heating on the extent of non-specific binding of fluorophore-labeled target DNA. Fig. 5 shows the results for the model assay shown in Fig. 2 after both room temperature (bottom) and microwave incubation (Mw) for 20 s (top).

Remarkably, the same fluorescein emission intensity [*ca.* 3800 arbitrary units (A.U.)] is observed after just 20 s, as compared to a 3.5 h incubation, which was predetermined to be the time required for the emission signal to change by >95% of its final value. These spectra were the mean of three separate surface measurements, which were all of very similar intensity. This result is most encouraging and suggests that the use of low-power microwaves has great utility for DNA target hybridization assays, where a >600-fold decrease in assay run time was observed in this sample alone, *i.e.* 20 s *versus* 3.5 h. In Fig. 5, the sample surface was pretreated with succinic anhydride, which we have previously shown to help block the extent of non-specific absorption of DNA to the amine-coated, *unsilvered* glass portion of the slide support, due to surface charge repulsion.[25] Interestingly, when the samples were not coated with the complementary surface-thiolated oligo, but were still NH_2-blocked, the extent of non-specific absorption was reduced 50% after microwave exposure as compared to room temperature incubation, *cf.* Fig. 5(bottom; dashed curve) and Fig. 5(top; dashed curve). This suggests that the method of assay heating has some influence on both the rates and extent of non-specific assay absorption. To the best of our knowledge, this is the first observation of microwave heating reducing the extent of non-specific absorption, and will consequently be discussed in further detail later. Fig. 5 inserts

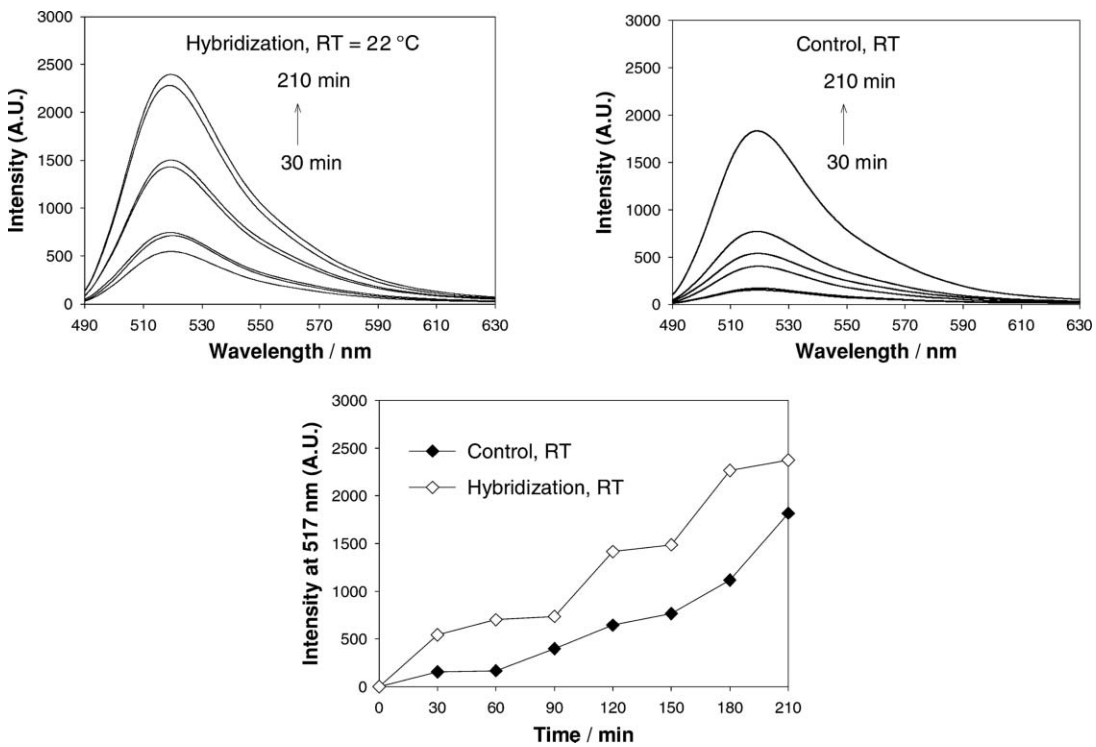

Fig. 4 Emission spectra of fluorescein-DNA (3 μM) as a function of hybridization time at 22 °C (top, left); emission spectra of a control sample which contains no thiolated oligo on the SiFs (top, right); and the respective intensity values *vs.* incubation time (bottom). The surfaces were NH_2-blocked (succinic anhydride) as described in the Experimental section.

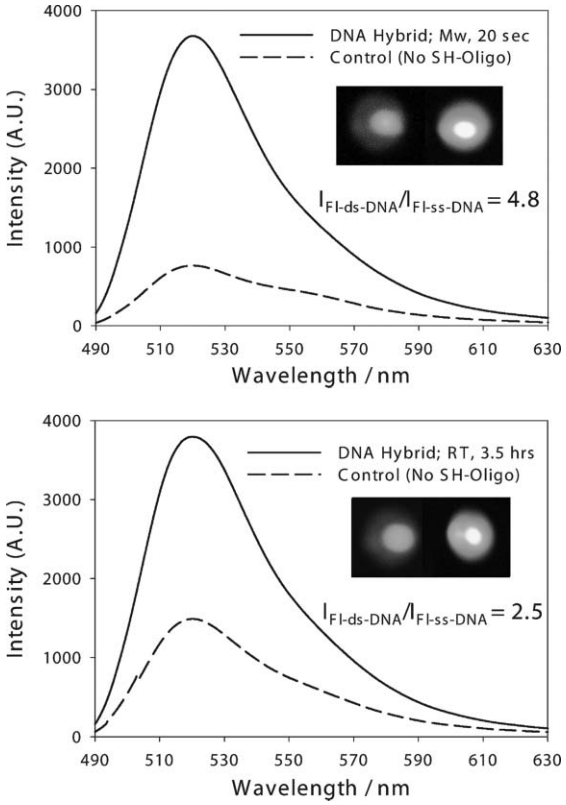

Fig. 5 Emission spectra of fluorescein-oligo (3 μM) after microwave-accelerated hybridization on SiFs (top), insert – photographs both before and after microwave (Mw) heating. (Bottom) Emission spectra and insert – photographs, both before and after room temperature hybridization. The measurements were the mean spectra of three separate surface locations. The surfaces were NH_2-blocked (succinic anhydride) as described in the Experimental section.

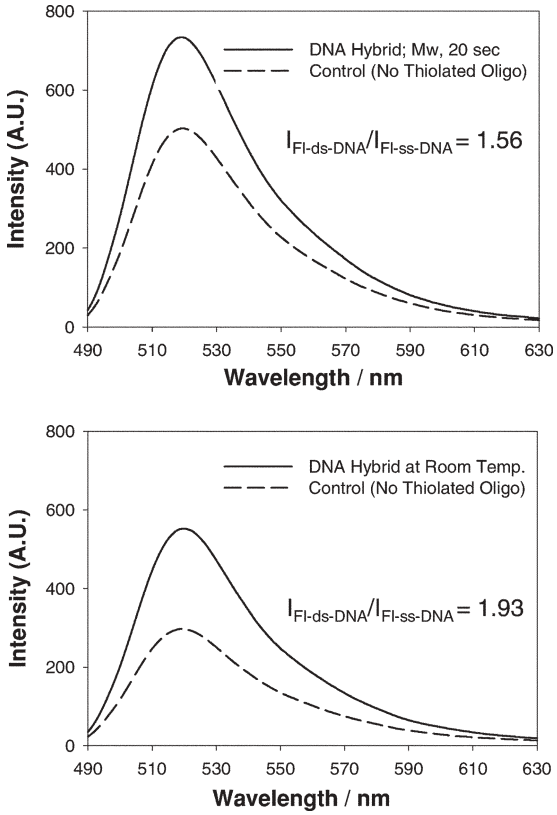

Fig. 6 Emission spectra of fluorescein-oligo (3 μM) after both 20 s microwave heating (top) and 3.5 h room temperature incubation (bottom). The measurements were the mean spectra of three separate surface locations. In both experiments the surfaces were *un-blocked*.

show the photographs of the fluorescein emission from the 50 μl black body wells, taken through the same emission razor edge filters as were used to collect the emission spectra in Fig. 5. Clearly, a much brighter fluorescence emission is observed from the silvered portion of the glass slides, consistent with our numerous reports of enhanced fluorescence signatures from fluorophores close to metallic nanostructures.[13]

To demonstrate the importance of blocking the glass surfaces, we also repeated the same experiment using glass surfaces which were un-blocked and not pretreated with succinic anhydride. As expected, the overall emission intensity ratios (intensity of Fl-ds-DNA/intensity of Fl-ss-DNA due to non-specific absorption) fell dramatically, from 4.8 [Fig. 5(top)] to 1.56 [Fig. 6(top)] for the microwave-heated assay, both with and without surface NH_2-blocking and surface capture thiolated oligo. For the room temperature incubated assay, the emission intensity ratios fell from only 2.5 to 1.93, *cf.* Fig. 5(bottom) and Fig. 6(bottom) respectively. This result suggests that the glass NH_2-surface blocking with succinic anhydride had only a minor effect for room temperature incubated samples for 3.5 h, but a major effect on microwave-accelerated assay samples. This effect is thought to be due to the fact that an increased length of time is needed to facilitate unwanted, non-specific DNA absorption during long incubation times, supporting the notion that reduced unwanted background absorption can be achieved using microwave-accelerated heating. It should be noted that the differing *y*-axis intensity values of Fig. 5 and Fig. 6 are arbitrary, and simply reflect laser power differences and respective sample alignments on the TIRF stage (not shown).

It is also interesting to comment on the overall extent of non-specific absorption in Fig. 5 for blocked surfaces. In Fig. 5(top) a *ca.* 21% extent of non-specific absorption can be observed, even though the glass surface is NH_2-blocked. In contrast a *ca.* 64% extent of non-specific absorption can be seen when the surfaces are un-blocked, Fig. 6(top). Therefore, one could rationale that *ca.* 40% non-specific absorption can be accounted for by blocking the glass surface with succinic anhydride. The 21% of signal attributed to non-specific absorption in Fig. 5(top) is subsequently thought to be due to the non-specific absorption of the DNA to the silver islands themselves, noting that the succinic anhydride blocks *only the surface glass NH_2-groups*.

In this regard numerous reports have reported the absorption of ss-DNA to metallic particles.[12,26,27] While more elaborate surface chemistries can be envisaged to further alleviate non-specific DNA absorption to SiFs, the focus of this paper is to demonstrate the MAMEF concept when applied to DNA target detection, as compared to the development of a fully optimized assay. This issue will be further addressed in a future manuscript by our laboratories.

Given that the use of low-power microwaves in DNA hybridization assays reduces the non-specific binding as shown in Fig. 5, next, we investigated this phenomenon for a range of DNA concentrations for the control experiments. Fig. 7 shows a non-specific binding comparison of the peak emission intensity of fluorescein-DNA after both room temperature and microwave heating in the absence of any SH-DNA surface anchors. A detailed examination of Fig. 7 reveals that the extent of non-specific absorption was reduced up to 50% after microwave exposure as compared to room temperature incubation, especially for the larger DNA concentrations. This suggests that the microwave heating has a significant influence on the extent of non-specific assay absorption in control experiments. It is also important to note that a reduction in non-specific binding in the control experiments directly translates into better sensitivity in the DNA hybridization assays.

Effects of microwave heating on DNA

As briefly mentioned in the Introduction, previous work by our laboratories has shown that low-power microwaves do not denature or perturb protein conformation.[17] In an analogous manner, we have considered the effects of low-power microwave heating on the ability of DNA to both melt and re-hybridize with further complementary target. Fig. 8 shows the emission spectra of fluorescein-DNA after 20 s microwave-induced hybridization (top), after melting the DNA and removing the fluorescein-ss-DNA (middle) and after re-hybridization with fresh fluorescein-ss-DNA using 20 s microwave heating (bottom). As we can see, the fluorescein fluorescence intensity is retained (*ca.* 600 A.U.) after re-hybridization (bottom), strongly suggesting that the DNA is unaltered during low-power microwave heating. This finding suggests that future surfaces made for MAMEF-based assays can be made both reusable and reversible for DNA target sensing applications.

While not shown here, we have also found that low-power microwaves can induce not only hybridization during rapid temperature jumps, but they can also induce melting at much longer microwave exposure times (appropriate higher temperatures). This suggests the development of *reusable*, simple, low-cost and low-power microwave devices which contain metallic nanoparticles, for the ultra-sensitive and ultra-fast detection of DNA, away from a laboratory, such as in field-deployable devices and test strips.

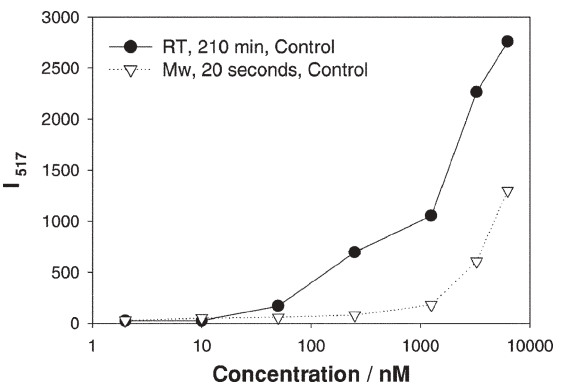

Fig. 7 A non-specific absorption comparison of the peak emission intensity of fluorescein-DNA after both room temperature and Mw annealing in the absence of any SH-DNA surface anchors. The surfaces were NH_2-blocked (succinic anhydride) as described in the Experimental section.

Surface temperature jump using low-power microwave heating

The temperature jump in the entire solution above SiFs (bulk) during low-power microwave heating was previously investigated by employing a thermally responsive fluorophore, Acridine Yellow (AY).[18] In that study, the temperature-dependent emission spectrum of AY in solution was used to plot a calibration curve, *i.e.* normalized intensity (the intensity at any time divided by the initial intensity(*versus* the temperature (20–70 °C). Subsequently, the temperature change in bulk due to microwave heating was determined using the calibration curve.

In the present study, complementary to our previous observations in bulk, we furthered our understanding of the surface temperature increase due to microwave heating by investigating the temperature change in close proximity to the SiFs. This was accomplished by employing evanescent wave excitation that only penetrates *ca.* 200 nm above the glass–liquid interface.[21] The temperature localized to SiFs was determined from the temperature-dependent spectra of AY exposed to microwaves for up to 30 s (measured simultaneously while heating in the microwave cavity) by comparing the normalized intensity at 506 nm *vs.* the microwave heating time[18] with those values in the calibration curve mentioned above. The temperature localized around the SiFs was determined to be 23 °C [using evanescent wave geometry, Fig. 1(left)] while the initial solution temperature was 22 °C.

It is important to discuss the reasons for such increases in temperature for a system comprised of metal colloids and water. It was previously shown that the microwave energy both absorbed and focused by metal colloids is dissipated local to the colloids, and that this energy dissipation contributes minimally to bulk heating.[28] The temperature dissipated around the gold colloids after microwave exposure was shown to be in the order of a few microKelvin.[28,29] Thus, the primary source of increase in the *bulk temperature* was concluded[18] to be due to the absorption of energy at 2.45 GHz directly by water, which we believe probably accounts, to some extent, for the faster hybridization kinetics. As shown in Fig. 8, such a rapid acceleration in temperature did not denature DNA and thus the microwave-accelerated hybridization assays using silver colloids were made possible.

While metallic nanoparticles dissipate only a very small amount of heat (microKelvin), Finite-Difference Time-Domain (FDTD) calculations indicate that there is also a focusing of microwaves around surface-bound structures. Consistent with this finding, we have recently reported and simulated metal-enhanced and 'triggered' chemiluminescence from surface structures.[30] Subsequently, it is believed that this localized focusing above the particles rapidly and locally heats the water/buffer, facilitating the very fast hybridization kinetics which cannot be explained by a bulk temperature jump of only a few degrees C. Contradictory to this are our

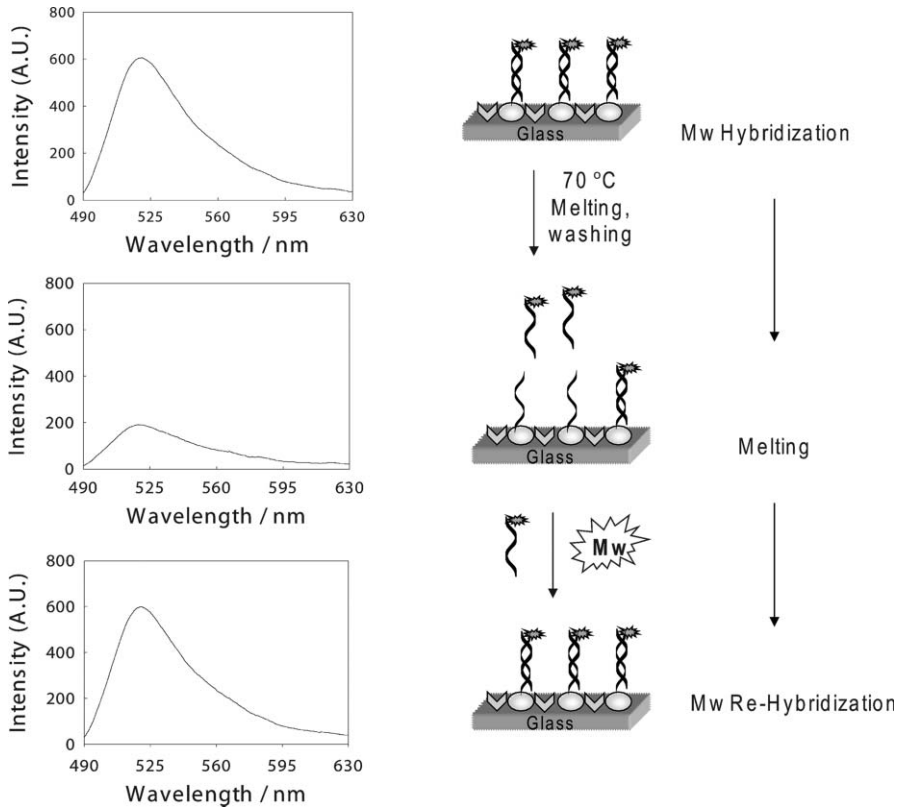

Fig. 8 Emission spectra of Fl-DNA after hybridization (top); after melting at 70 °C (middle) and after a further 20 s low-power Mw heating period with an additional 250 nM Fl-DNA (bottom). The silvered surface was washed with buffer several times between each measurement.

observations that the bulk temperature is higher than that probed local to the surface, using evanescent wave excitation. In this geometry we excite <200 nm of solution above the metal.[21] Even though this depth of solution is small as compared to the *ca.* 1 mm thick bulk, it is thought that the focused microwave volume region is even smaller than this, the emission of AY from this region contributing but only a small fraction to the probed volume.

In summary, three forms of heating are believed to occur:

(1) Heating of the metal directly (ohmic heating), this is then dissipated into solution.

(2) Direct heating of the bulk solution.

(3) Field-enhanced heating around the nanoparticles, resulting in an increased mass DNA transport to the surface.

Advantages of the MAMEF DNA sensing platform

The MAMEF-based DNA detection methodology described here has several notable advantages over current DNA detection technologies,[1,5] including:

• The fluorescence amplification occurs due to the close proximity of fluorophores to the silver nanostructures, which occurs upon DNA hybridization. In most DNA assays to date,[1] fluorophore spectral changes are a function of probe intercalation within the ds-DNA and not surface proximity, as demonstrated here.

• The fluorescence amplification provided by the silver has been shown in many reports by our laboratories to be applicable to many fluorophores and therefore wavelengths, from the UV to the NIR.[14,31] Hence fluorophores currently used in assays would also be suitable for use with MAMEF-based DNA sensing. In addition, the use of low quantum yield fluorophores would be most desirable, as these would not only lead to significantly larger fluorescence enhancements, *i.e.* $1/Q_0$,[32] but one could easily discriminate against unwanted background signal from low quantum yield fluorophores distal from the metal, recalling that MEF is a close range (4–10 nm) through-space interaction.

• The low-power microwaves here do not perturb the silver nanostructures or produce 'arcing' or 'sparking'.[17] Instead, the nanostructures localize the heating, facilitating both the MEF effect and heating in close proximity to the silver.

• We have shown that the MEF phenomenon can provide for increased emission intensities up to several thousand-fold for fractal-like surfaces.[24,33] This is likely to substantially increase the detection limits for DNA hybridization-based assays, and can be used as appropriate for assay platforms.

• The MAMEF DNA sensing platform could similarly be applied to RNA target base sensing for the rapid and sensitive detection of RNA-based diseases such as Avian Influenza and Ebola virus. In this regard, our laboratory has recently reported[23] a simple three-piece RNA target-based MEF assay. While the assay does not use low-power microwaves for rapid kinetic acceleration, the MEF-based substrate and assay design readily provides for femto-molar 500-mer RNA sensitivity. Our MAMEF DNA platform could therefore simply be applied to RNA target detection also.

• A whole range of surfaces can be routinely prepared on either glass[13] or plastic supports,[22,34] which do not require the benefits of a nanofabrication laboratory. These surfaces offer a range of MEF-based enhancements for both one-[13] and multi-photon excitation.[35]

• The reduced lifetimes of fluorophores in close proximity to silver readily provide for enhanced fluorophore photostability.[13,14] In addition, shorter lifetimes allow for higher fluorophore cycling rates,[36] further providing for increased fluorophore intensities and therefore assay detectability.[36]

• The low-power microwaves used here do not cross-link 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, *cf.* Fig. 8.

• The use of low-power microwaves to kinetically accelerate assays significantly reduces both the rate and extent of non-specific absorption. This is a *significant finding* as the sensitivity (analytical detectability) of many assays is governed by the overall extent of non-specific absorption.

Conclusions

In this paper, we have provided the detailed results for the investigation of the effects of low-power microwave heating on a MEF-based surface DNA sensing platform that could be applied to many other DNA or RNA targets. Our approach has the possibility of being easily applied to DNA chips or even multi-well plates for the multiplexed multicolor simultaneous detection of many DNA/RNA targets. This approach provides for both ultra-fast and ultra-bright DNA assays to be realized, an area of intense clinical-, environmental- and military-based research.

Our model hybridization assay combines the use of nanometer-sized silver particles, which dramatically increase the emission intensity of close-proximity fluorophore labels with the use of low-power microwave heating to rapidly and uniformly heat the assay. The microwaves do not perturb the silver nanostructures, cause sparking or arcing, or even perturb the local DNA. Instead the rapid heating local to silver affords for a dramatic increase in the mass DNA transport to the surface. Subsequently in our model system, MAMEF-based DNA detection provides up to a 600-fold reduction in the assay run time, with a greater than ten-fold increase in assay sensitivity. It was also found that low-power microwave heating significantly reduces the non-specific binding of target DNA, which affords an even further increase in the sensitivity of the MAMEF-based DNA hybridization assays. These findings are likely to be of *major significance* for DNA-based detection, especially when speed and sensitivity are of the essence, such as in the identification of bioagents, such as for Anthrax (DNA) or even the Ebola virus (RNA). Work is currently underway in our laboratories in this regard and will be reported in due course.

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