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Fast and sensitive DNA hybridization assays using microwave-accelerated metal-enhanced fluorescence ☆

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Abstract

A new, fast, and sensitive DNA hybridization assay platform based on microwave-accelerated metal-enhanced fluorescence (MAMEF) is presented. Thiolated oligonucleotide anchors were immobilized onto silver nanoparticles on a glass substrate. The hybridization of the complementary fluorescein-labeled DNA target with the surface-bound oligonucleotides was completed within 20 s upon heating with low-power microwaves. In addition, the signal is *optically amplified*, a consequence of close proximity of the fluorophore to the silvered substrate. In this proof-of-principle methodology, as low as 50 nM of a target DNA was detected, although we envisage far-lower detection limits. Control experiments, where the surface-bound oligonucleotide was omitted, were also performed to determine the extent of non-specific binding. In these studies a significantly reduced non-specific adsorption was found when using microwave heating near to silvered structures as compared to room temperature incubation. These findings suggest that MAMEF could be a most useful alternative to the DNA hybridization assays used today, especially with regard to substantially increasing both the assay rapidity and sensitivity.

Keywords: Microwave-accelerated metal-enhanced fluorescence; Metal-enhanced fluorescence; DNA hybridization; Silver nanoparticles; Surface-enhanced fluorescence; Radiative decay engineering

Detection of DNA hybridization is the basis of a wide range of biotechnology and diagnostics applications [1] and is routinely used on gene chips [2,3], during PCR [4,5] and fluorescence-based in situ hybridization [6]. In most cases, the DNA hybridization 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, such as *unwanted* background emission and the photostability of the fluorescent probe used.

Recently, Malicka et al. [7] introduced an approach to DNA hybridization assays based on metal-enhanced fluorescence (MEF), where the change in fluorescence intensity allows one to track the hybridization of a complementary target on a solid surface. In that study, an increase of ≈12-fold in fluorescence intensity from the DNA hybridization assay on silver island films was obtained as compared to the fluorescence intensity from the same assay but in free solution. In addition, the photostability of the tagging fluorophore was significantly improved. These achievements were possible due to the MEF phenomenon, which is rapidly becoming a powerful tool for fluorescence-based assays [8–10].

In recent years our laboratories at the University of Maryland Biotechnology Institute have demonstrated many applications of metal-enhanced fluorescence [8–10], where

☆ *Abbreviations:* MAMEF, Microwave-Accelerated Metal-Enhanced Fluorescence; MEF, Metal-Enhanced Fluorescence; RDE, Radiative Decay Engineering; SiFs, Silver Island Films; AY, Acridine Yellow.

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the origins of MEF can be traced back to several groups working in this area [11,12]. In all of these applications of MEF it has been shown that the enhanced fluorescence signals (System quantum yields—Q_m) of fluorophores in close proximity (<10 nm) to metallic nanostructures could be well described by the following equations:

$$Q_m = (\Gamma + \Gamma_m)/(\Gamma + \Gamma_m + k_{nr}) \quad (1)$$

where Γ is the unmodified radiative decay rate, Γ_m is the metal-modified radiative decay rate, and k_{nr} are the non-radiative rates. Similarly, the metal-modified system lifetime, τ_m, for fluorophores is decreased by an increased radiative decay rate, effectively depopulating the S_1 state quicker:

$$\tau_m = 1/(\Gamma + \Gamma_m + k_{nr}) \quad (2)$$

These equations have resulted in most unusual predictions for fluorophore-metal combinations, and it is these predictions and observations that are currently finding profound implications and applications in fluorescence-based nanotechnology [8–10,13]. From Eqs. (1) and (2), we can see that as the value of Γ_m increases, the quantum yield Q_m increases, while the lifetime, τ_m, decreases. This is contrary to most observations in fluorescence [14] where the free-space quantum yield, Q_0, and lifetime, τ_0, usually change in unison as described by the well-known equations [14]:

$$Q_0 = \Gamma/(\Gamma + k_{nr}) \quad (3)$$

$$\tau_0 = 1/(\Gamma + k_{nr}) \quad (4)$$

A new platform technology, microwave-accelerated metal-enhanced fluorescence (MAMEF), which couples the benefits of MEF with the use of low power microwaves to kinetically accelerate bioaffinity reactions was recently introduced for assays [15] and immunoassays [16]. 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 within seconds, significantly reducing a bioassays' run time. Therefore, MAMEF provides for ultrafast and ultrabright immunoassays to be realized.

In this paper, the application of the MAMEF technology for ultrafast and sensitive DNA hybridization detection is presented. By combining the effects of silver nanoparticles on fluorophores with low power localized microwave heating, a model DNA hybridization assay, over the concentration range of 50–1250 nM, was kinetically completed within 20 s. The hybridization kinetics were accelerated over 60-fold, as compared to an identical hybridization assay run at room temperature. The microwave-induced temperature jump in the bulk medium was determined to be ≈5 °C from studies using a thermally responsive fluorophore.

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 SiFs onto glass slides was performed as described previously [15]. In a typical SiFs preparation, a solution of sodium hydroxide and ammonium hydroxide are added to a continuously stirred solution of silver nitrate at room temperature. Subsequently, the mixture is cooled down in an ice bath, Silane-prep™ glass slides are inserted, and a solution of D-glucose is added. As the temperature is increased, the color of the mixture turns yellow–brown and the SiFs-deposited slides are removed from the mixture, washed with water, and sonicated for a few seconds at room temperature. SiFs-deposited glass slides were stored in deionized water until they were used.

The preparation of DNA capture assay slides. First, in order to prevent the non-specific binding of oligomers on the part of the glass slides not covered by silver nanoparticles, all free glass surface amino groups were blocked with 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. SiFs-deposited glass slides were then coated with black electrical tape, which is attached to a self-sticking paper, containing three 5 mm wide circular holes (referred to as a "black body") on both the silvered and unsilvered slide, prior to the assay fabrication and subsequent fluorescence experiments.

Five hundred nanomolars of thiolated oligomer (5′-TCC-ACA-CAC-CAC-TGG-CCA-TCT-TC-(SH)-3′) was incubated overnight at 4°C on the surface of SiFs-deposited glass slides in Hepes Buffer (5 mM Hepes, pH 7.5, with final concentrations of 100 mM KCl, 0.25 mM EDTA, this buffer was used in all the experiments unless otherwise stated) followed by rinsing with water to remove the unbound material, Fig. 1. The thiolated oligomer was covalently linked to SiFs via well-established self-assembled monolayer chemistry [17].

Microwave-accelerated metal-enhanced fluorescence (MAMEF)-based DNA capture assay using a microwave cavity. The MAMEF-based DNA capture assay was performed by the incubation of 30 μl of varying concentrations (2–1250 nM) of Fluorescein-labeled oligomer (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). In order to determine the extent of non-specific binding of Fluorescein-labeled oligomer, the Fluorescein-labeled

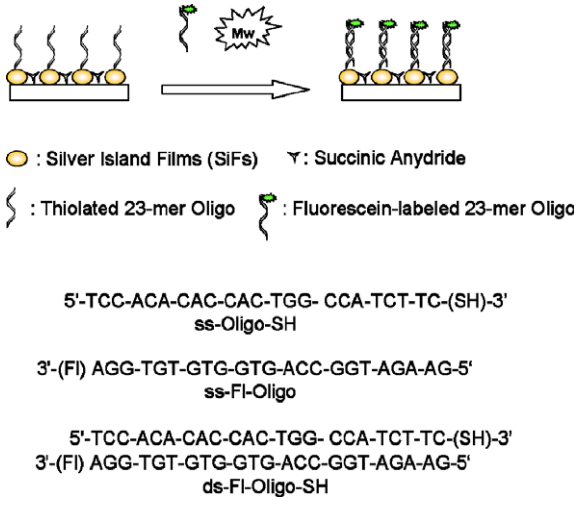

Fig. 1. Microwave-accelerated metal-enhanced fluorescence-based sensing scheme for fast and sensitive DNA target detection. The lower panel shows the structures of the DNA oligomers used in this study.

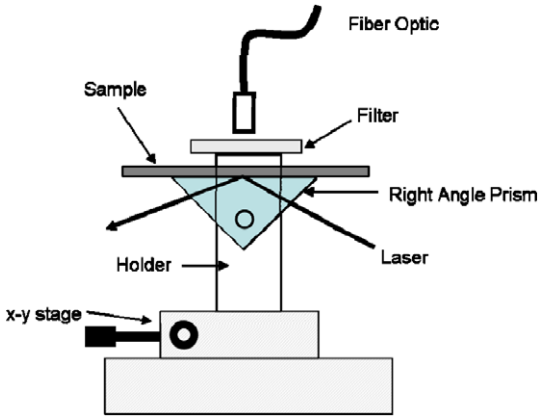

Fig. 2. Total-internal reflection fluorescence (TIRF) geometry for DNA hybridization assays.

oligomer was incubated on succinic anhydride blocked SiFs surface, in the absence of immobilized thiolated oligomer for 20 s in a microwave cavity. The power setting of the microwave cavity was set to 2 which corresponded to 140 W over the entire cavity. This power is similar to the numerous reports using low power microwaves for immunolabeling [18], Immunostaining [19,20] in immunocytochemistry [21,22], and histological microwave processing [23,24]. In all the experiments performed with low power microwaves, using both glass slides and quartz cuvettes modified with the "black body", there was no evidence of surface drying.

Temperature calibration in the microwave cavity. In order to calibrate the temperature jump during low power microwave heating, a thermally responsive fluorophore, Acridine yellow (AY), was employed. First, the temperature-dependent emission spectrum of AY was recorded in bulk solution using a fluorometer (Varian) as the temperature was gradually increased from 20 to 70 °C using the single cell Peltier accessory. The temperature-dependent spectra were used to plot the calibration curve, i.e., normalized intensity (intensity at any time divided by the initial intensity) versus the bulk solution temperature. The change in temperature of the solution above the SiFs, due to microwave heating, was determined from the temperature-dependent spectra of AY (on the samples that were placed inside the cavity) when microwave heated for up to 30 s. The emission spectra of AY on SiFs (while they remained inside the cavity) were recorded with a front-face geometry, whereby AY is excited at 45° and emission is detected at 90°. The temperature of the solution on the SiFs after the microwave heating was determined by comparing the normalized intensity with those values in the pre-determined calibration.

Fluorescence spectroscopy. All fluorescence assay measurements were performed by collecting the emission intensity through a long pass filter (488 nm) perpendicular to the assay surface, after total-internal reflection evanescent wave excitation, using a 473 nm diode laser and a Fiber Optic Spectrometer (HD2000) from Ocean Optics, Inc., Fig. 2.

Results and discussion

The MAMEF-based DNA capture assay was performed by the incubation of 30 μl of varying concentrations (2–1250 nM) of Fluorescein-Oligo (ss-Fl-Oligo, Fig. 1) with a strand complementary to the thiolated oligo immobilized on SiFs for 20 s in a microwave cavity. The extent of non-specific binding of Fluorescein-Oligo was determined by repeating the same experiment but in the absence of immobilized thiolated oligo.

Fig. 3 shows the emission spectra of Fluorescein-Oligo as a function of concentration after 20 s low power microwave heating and also the emission spectra of Fluorescein-Oligo used in the control experiment where the surface-bound SH-Oligo anchor was omitted. The emission intensity of the Fluorescein-Oligo progressively increased as the concentration is increased both in the hybridization and control assays, as more Fluorescein-Oligo was available for the hybridization and for non-specific binding. Interestingly, the use of low power microwaves enabled the assay to be completed within a few seconds. This finding is similar to our recent report of ultrafast and ultrasensitive protein determination [15,16].

Fig. 4 shows a semi-logarithmic plot of fluorescence emission intensity at 517 nm of Fluorescein-Oligo for the hybridization and control assays as a function of target DNA concentration. The dynamic range of detection of the Fluorescein-Oligo is roughly found to be between 50 and 1250 nM, where the extent of non-specific binding could be seen to vary from 10% and 40%.

The inserts in Fig. 3 show the comparison between the background signal (SiFs, no oligomers) and the emission spectra of 2 and 50 nM Fluorescein-Oligo in the hybridization and control assays. The signal-to-noise ratio (S/N) at 517 nm was found to be larger than 3 for the hybridization assay, which is comparable to the range of S/N values typically acceptable for fluorescence-based assays [14].

The use of silver nanoparticles in MAMEF-based DNA hybridization assays has two main purposes: (1) silver nanoparticles deposited on the glass slides serve as a platform where the anchor probes can be incorporated onto glass slides: the anchor probe is immobilized to the surface of the silver via thiol chemistry. This scheme allows the hybridization of fluorophore-labeled oligo with the anchor probe to occur near to the silvered surface where the fluorescence emission is increased, i.e., MEF [8–10]; (2) silver nanoparticles increase the sensitivity of the hybridization assays, that is, a lesser amount of oligomers can be detected due to the increased emission and photostability. This is possible due to the metal-enhanced fluorescence phenomenon that results in increased fluorescence emission intensity and a decrease in lifetime of the fluorophores in close proximity to silver nanoparticles [8–10].

In a previous publication by Malicka et al. [7], it was shown that when a fluorophore-labeled oligo was hybridized with its complimentary target, that was immobilized onto silver nanoparticles, the emission intensity was increased ≈12-fold as compared to an equivalent amount of hybridized partners in solution. Moreover, the lifetime of fluorescein was reduced from 3.41 ns in solution to 0.18 ns on SiFs providing further proof for metal-enhanced fluorescence, cf. an increase in Q_m and simultaneous reduction in τ_m, cf. Eqs. (1) and (2). In this paper, we have significantly improved on that finding, the hybridization time being reduced from 20 min to 20 s (a 60-fold increase in the hybridization kinetics) for the same oligomer system that was used by Malicka et al. [7].

The much faster kinetics reported here have been 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

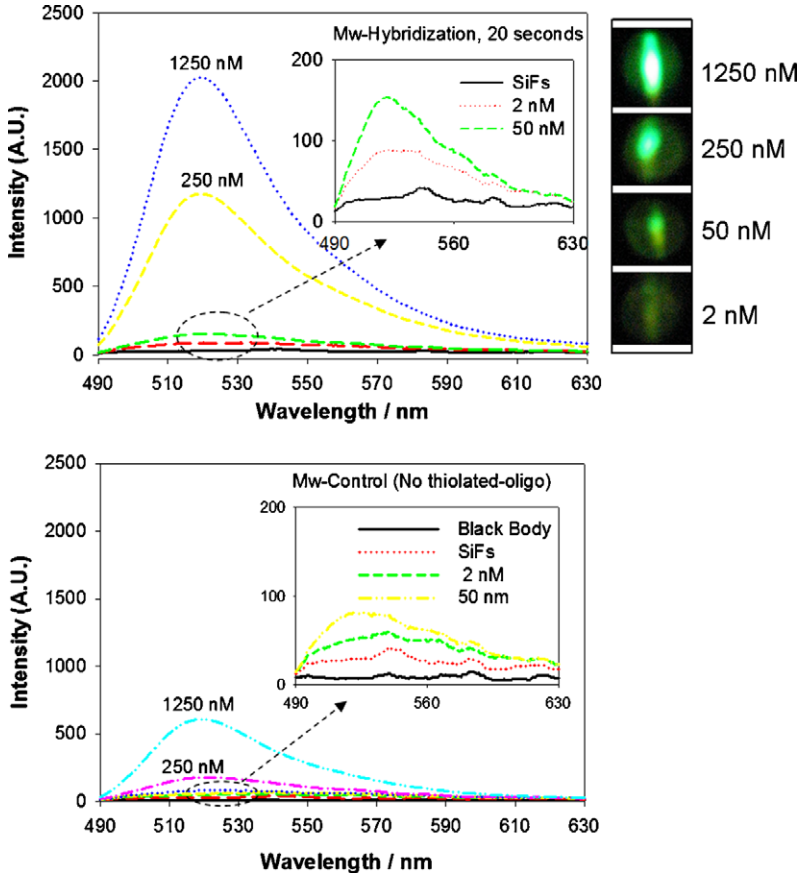

Fig. 3. Emission spectra of Fluorescein-Oligo as a function of concentration after 20 s low power microwave heating (top); photographs showing an increased emission intensity as a function of Fl-DNA concentration after 20 s Mw heating. Bottom–A control sample identical to the top panel but with no surface bound SH-Oligo anchor.

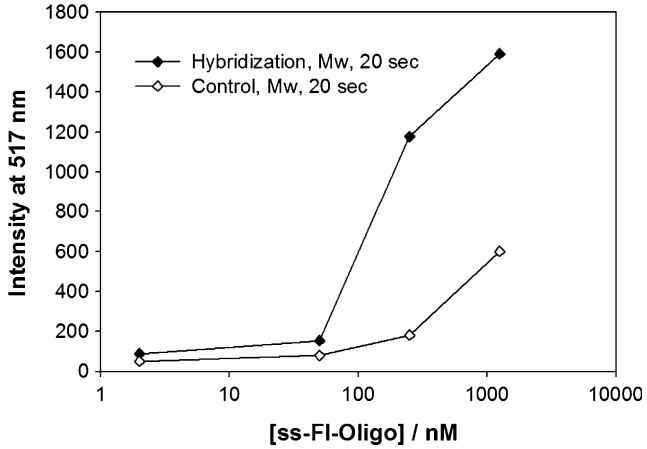

Fig. 4. Semi-logarithmic plot of the fluorescence emission intensity at 517 nm for the Fluorescein-Oligo as a function of concentration derived from Fig. 3.

conjunction with silver nanoparticles on the kinetics of the biomolecular interactions, such as protein-protein and antibody-antigen interactions, has been shown elsewhere by us [15]. 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 [25]. This leads to localized heating around the silver nanostructures in addition to the heated solvent, rapidly accelerating assay kinetics. Fluorescence lifetime and fluorescence resonant energy transfer studies have recently shown that microwave heating does not induce protein structural or environmental changes [15]. A detailed report of the effects of microwave heating on DNA hybridization will be reported in due course by us.

In order to investigate the temperature jump during low power microwave heating, a thermally responsive fluorophore, acridine yellow (AY), was employed. Fig. 5 shows the temperature-dependent emission spectrum of AY, as the temperature was gradually increased from 20 to 70 °C. As expected, the emission intensity of AY increased as the temperature increased. The temperature-dependent spectra, Fig. 5, were used to plot the calibration curve, i.e., normalized intensity (intensity at any time divided by the initial intensity) versus the temperature, Fig. 6-top.

The temperature change of the solution above the SiFs, due to microwave heating, was determined from the

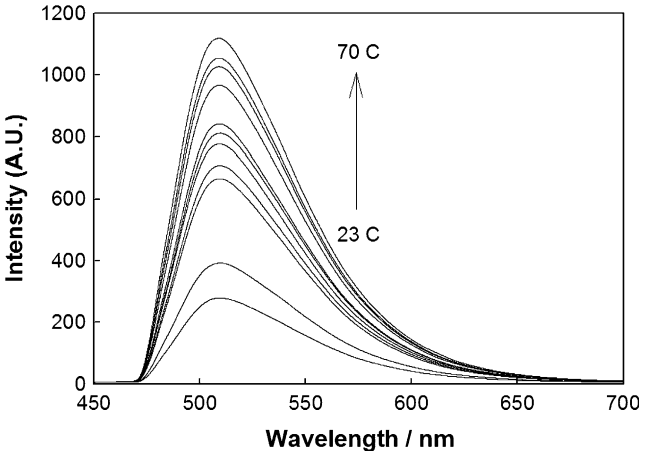

Fig. 5. Emission spectra of acridine yellow, AY (lex = 473 nm) in bulk solution as a function of temperature (heated with Peltier system).

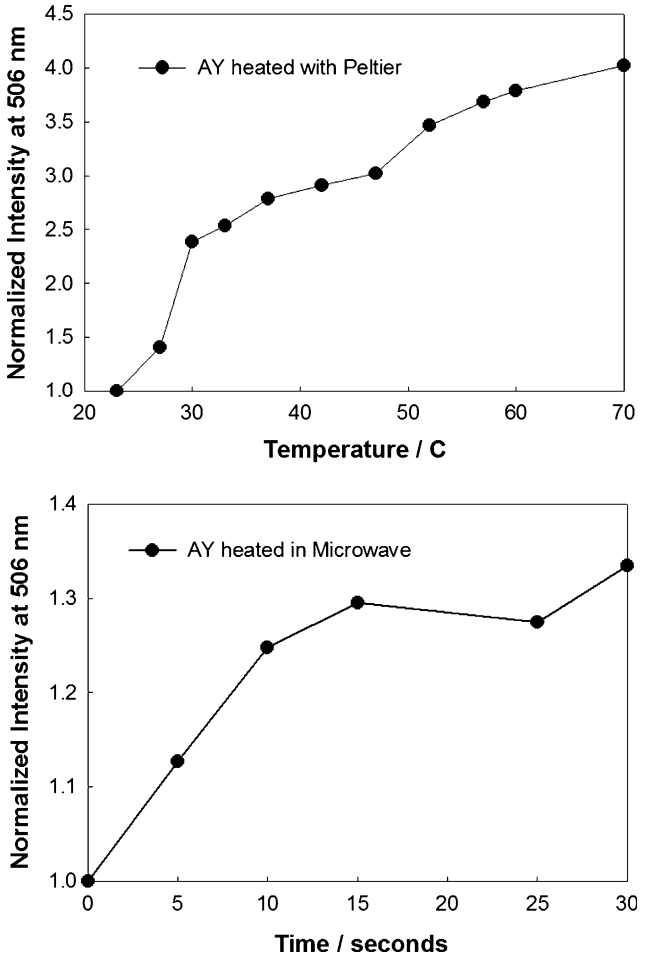

Fig. 6. Normalized intensity at 506 nm vs. temperature for AY bulk solution heated with Peltier system obtained from Fig. 5 (top) and the plot of the normalized intensity at 506 nm vs. time of microwave heating for AY on SiFs heated in a microwave cavity (bottom).

temperature-dependent spectra of AY exposed to microwaves for up to 30 s. This was done by comparing the normalized intensity at 506 nm vs microwave heating time (Fig. 6-bottom) with those values in the calibration curve that were obtained in Fig. 6-top. For a 20 s DNA hybridization assay, it is estimated that the temperature of the bulk medium above the SiFs increased from 23 to 28 °C. However, the increase in the temperature around the silver nanoparticles is believed to be much higher due to localized heating around/on the nanoparticles [15], which results in faster hybridization kinetics.

Conclusions

In this paper, we have demonstrated microwave-accelerated metal-enhanced fluorescence (MAMEF)-based DNA hybridization assays. A model DNA hybridization assay, which usually takes 20 min to reach completion at room temperature, was completed in less than 20 s (a >60-fold decrease in assay run time), with the use of low-power microwaves in combination with silver island films. With the new MAMEF technology, as low as 50 nM of DNA was detected in 20 s. Silver island films serve three purposes in the hybridization assays presented here: as a platform to immobilize the anchor probe on a solid surface; as an enhancer of the emission intensity; and to localize the heat delivery.

The MAMEF technology promises an ultra fast and ultra sensitive alternative to DNA target detection using relatively inexpensive equipment. Detailed studies to further understand the effects of microwaves on DNA are underway and will be reported shortly by our laboratory.

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