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ORIGINAL PAPER

Real-time Thermal Imaging of Microwave Accelerated Metal-Enhanced Fluorescence (MAMEF) Based Assays on Sapphire Plates

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Abstract In this paper, we describe an optical geometry that facilitates our further characterization of the temperature changes above silver island films (SiFs) on sapphire plates, when exposed to microwave radiation. Since sapphire transmits IR, we designed an optical scheme to capture real-time temperature images of a thin water film on sapphire plates with and without SiFs during the application of a short microwave pulse. Using this optical scheme, we can accurately determine the temperature profile of solvents in proximity to metal structures when exposed to microwave irradiation. We believe that this optical scheme will provide us with a basis for further studies in designing metal structures to further improve plasmonic-fluorescence clinical sensing applications, such as those used in microwave accelerated metal-enhanced fluorescence (MAMEF).

Keywords Immunoassays · Ultrasensitive assays · DNA detection · Low-Power microwaves · Metal-Enhanced Fluorescence (MEF) · Plasmons · Plasmonics · Microwave Accelerated Metal-enhanced Fluorescence (MAMEF) · Radiative decay engineering · Surface enhanced fluorescence · Plasmon enhanced fluorescence · Plasmon enhanced luminescence

Introduction

In recent years, we have demonstrated many new technological advances that combine microwave technologies and metal-enhanced fluorescence to improve the rapidity and sensitivity of clinical assays on silver island films [1–4]. In this work, we employed temperature sensitive fluorescent probes to determine temperature increases at the surfaces of these substrates and assays [1]. We also approximated the temperature increase of solvent in proximity to metal nanoparticles upon exposure to short 2.45 GHz microwave pulses [5].

Thermal or infrared imaging has been used to determine the temperature distribution for materials heated in microwave cavities [6, 7]. Many of these experiments are performed to compare the before and after temperature distributions in the material [8], but it is also possible to perform *online* thermal imaging to characterize temperature distributions of materials during exposure to microwave fields [6].

In this paper, we describe an optical geometry that facilitates our further characterization of temperature changes of island films (SiFs) on sapphire plates. Since sapphire transmits IR, we designed an optical scheme to capture real-time images of the temperature distributions of a thin water film on sapphire plates with and without SiFs during the application of a short microwave pulse (Fig. 1). From these results, we demonstrate that our previous experimental and theoretical estimates of the heating of water in proximity to silver island films correlate well with new thermal imaging data [1, 5]. Using this optical scheme, we can accurately determine the temperature profile of solvents in proximity to metal structures when exposed to microwave irradiation. We

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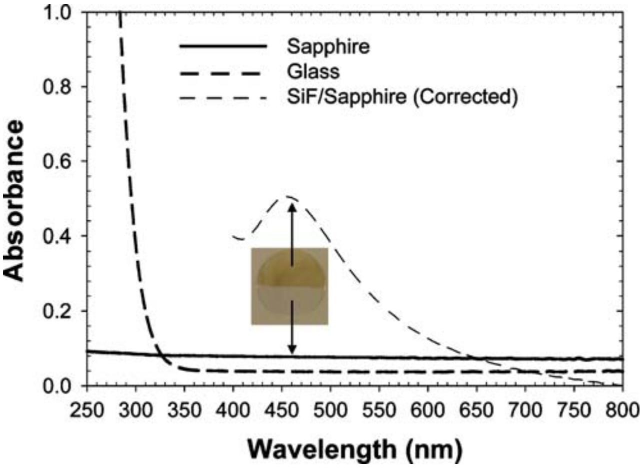


Fig. 1 Absorption spectra for sapphire (inset, bottom half), glass and SiF/Sapphire (inset, top half), which is baseline corrected with respect to the sapphire absorption spectra

believe that this optical scheme will provide us with a basis for further studies in designing metal structures to further improve plasmon coupled fluorescence clinical sensing applications.

Results and discussion

While our previous microwave-accelerated metal-enhanced (MAMEF) work was performed on glass substrates, [1] we demonstrate that the principles of MAMEF for a biotin/streptavidin assay extend to sapphire as well (Fig. 2). Sapphire substrates with and without silver island films were incubated with BSA-biotin to form a monolayer [1]. Subsequently, the treated sapphire plates were incubated with a solution of FITC-avidin for thirty minutes at room temperature (Fig. 2a) and for thirty seconds during exposure to 2.45 GHz microwave irradiation (Fig. 2b) [1]. Using microwave irradiation and sapphire plates, we reproduced the results from our previous report of MAMEF on glass substrates, whereby we substantially decreased the incubation time required for maximum streptavidin/biotin complex formation and increased the fluorescence detectability due to the MEF effect [1].

Since sapphire transmits IR radiation, it is an ideal substrate for thermal imaging experiments (IR transmission for camera 2-4.8 mm). Two hundred microliters of water was sandwiched between two sapphire plates (Fig. 3a, left). For SiF imaging experiments, the top sapphire plate was modified with silver island films (Fig. 3a, right). With this configuration, we could determine the average temperature increase of the water in proximity to the silver island films. The optical configuration consists of a microwave cavity with a small 1 inch diameter opening at the base, a gold mirror, and a thermal imaging camera (Silver 420 M; Electrophysics Corp, Fairfield, NJ) that is equipped with a close-up lens that provides a resolution of approximately 300 μm (Fig. 3b).

The clear sapphire plate of the sandwich geometry is fixed to the base of the microwave cavity opening. A gold mirror is positioned such that the image of the opening is reflected into the thermal camera. Thermal imaging data is recorded at 100 frames/second before, during and after the application of a fifteen second train of microwave pulses. Timing graphs are reflective of the mean intensity temperature over 100×100 pixel^2 region for the thermal images.

From mean temperature versus time plots for sapphire and sapphire/SiFs sample geometries, we observed slightly higher mean temperature increases for the water on the sapphire/SiF substrates (Fig. 4a). Thermal images show the temperature distribution of the sapphire and sapphire/SiF sample geometries (Fig. 4b,c) at a discrete time point (Fig. 4b,c—dashed line). In addition, we note that we observed increased cooling rates on the silver island films

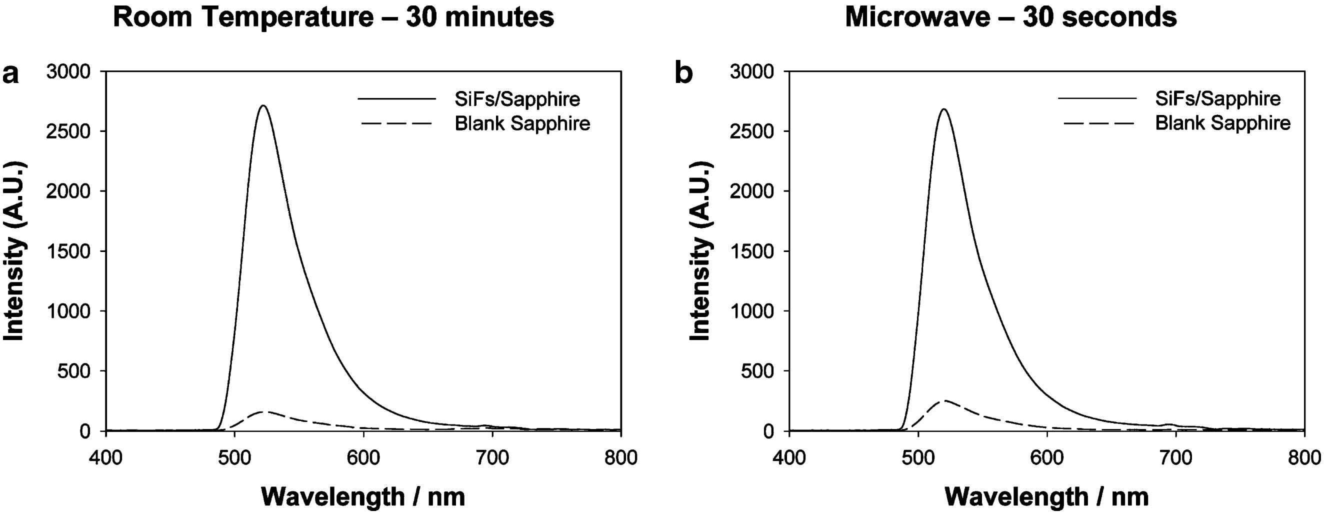


Fig. 2 Microwave accelerated metal-enhanced fluorescence (MAMEF) FITC-avidin/biotin-HSA surface assay on sapphire plates incubated for: **a** 30 min at room temperature and **b** 30 s during exposure to microwave pulse

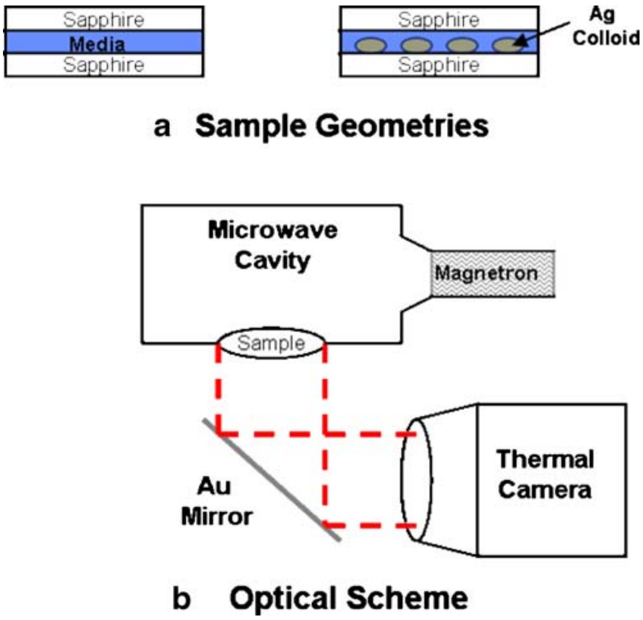


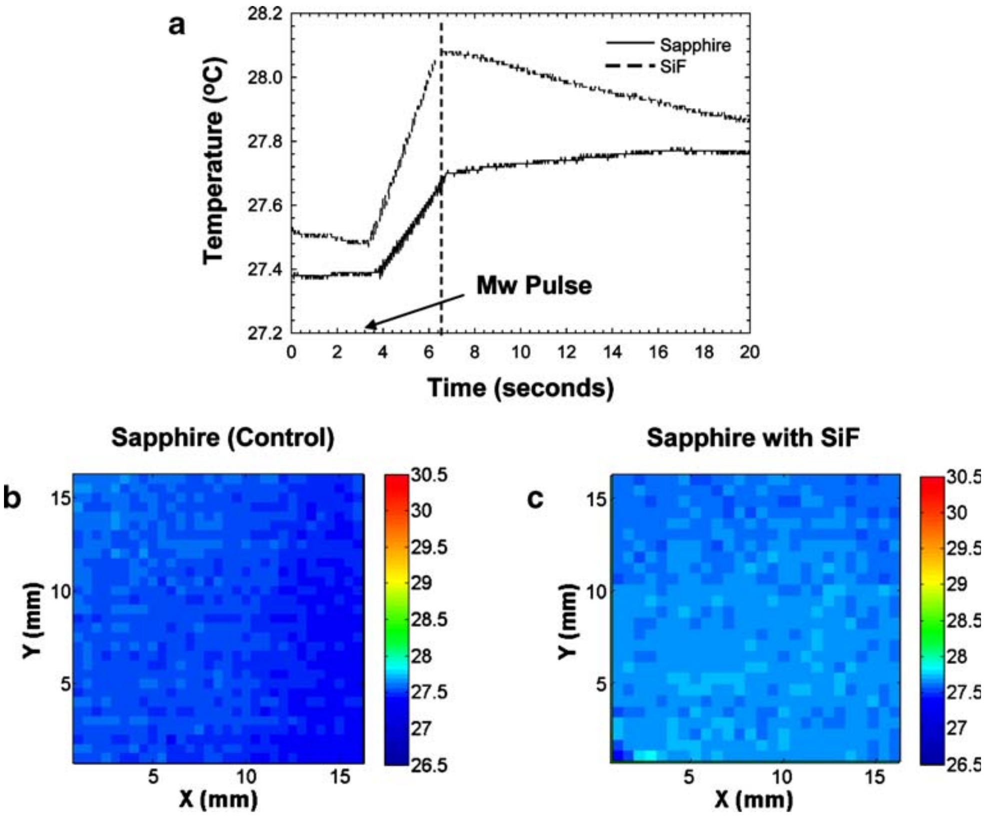
Fig. 3 (**a**) Sample geometries for blank sapphire and SiFs/sapphire thermal imaging experiments, where the sandwiched media is water. (**b**) Optical scheme for thermal imaging of SiFs and sapphire sample geometries

substrates. The observed mean temperature increases on SiF were consistent with predictions from our previous reports of the temperature rise of solutions on SiF/glass substrates upon exposure to 2.45 GHz microwave irradiation [1, 5]. While we note that the mean increase in temperature at the surfaces of these SiFs/sapphire substrates likely corresponds to increased collisional frequency of free FITC-avidin molecule with bound BSA-biotin, we believe the increased cooling rates in proximity to the metal substrates, which have higher thermal conductivities, contributes to a rapid *decrease* in Brownian motion upon collision or interaction at the *surface* of the metal colloids. As a result, we believe that both the increases in the heating rate in the bulk solution above the metal and cooling rate at the surface facilitates a decrease in non-specific absorption of the assay reagents upon exposure to microwave irradiation.

Conclusions

In conclusion, we have demonstrated that it is possible to thermally image water in proximity to sapphire/SiF substrates in real-time during the application of microwave irradiation. From mean temperature versus time plots, we correlate the mean maximum temperature change recorded using thermal imaging with our previous experimental and theoretical estimates of the heating of water in proximity to silver island film [1, 5]. Using this optical scheme, we provide a means to record real-time temperature distributions of solvents in proximity to sapphire surfaces modified with metal structures. By combining thermal imaging with

Fig. 4 **a** Time dependent thermal plots of average pixel intensity over 100 pixel2 region for SiF/sapphire and sapphire sample geometries where water is the sandwiched media. Real-time temperature distributions of water on **b** sapphire and **c** sapphire with silver island films



optical imaging in a microwave cavity, we hope to provide an optical configuration to better correlate increased bulk heating and surface cooling rates structures with reported increased specificity and rapidity of biomoleculer interactions exposed to microwave radiation [9].

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