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Faraz A. Choudhury, Grzegorz Sabat, Michael R. Sussman, Yoshio Nishi, and J. Leon Shohet

Citation: Journal of Vacuum Science & Technology A **33**, 061305 (2015); doi: 10.1116/1.4930315
View online: http://dx.doi.org/10.1116/1.4930315
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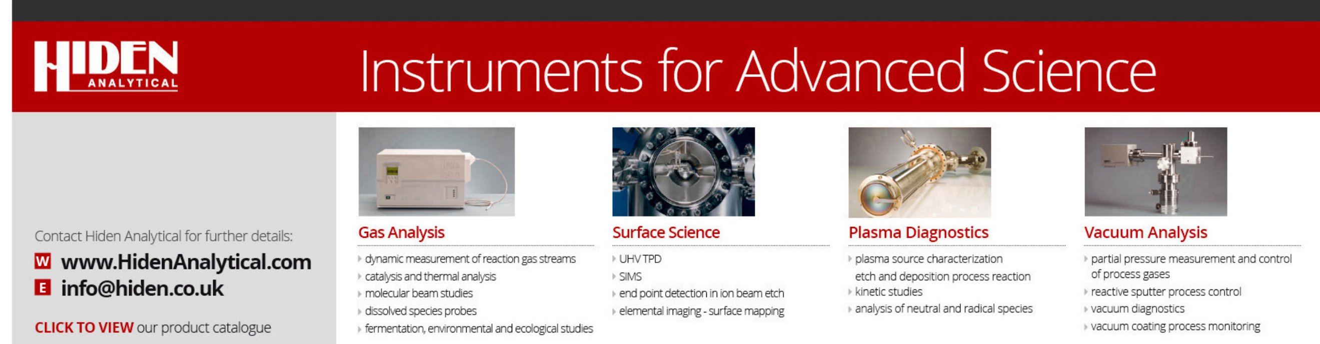

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Fluorophore-based sensor for oxygen radicals in processing plasmas

Faraz A. Choudhury
Plasma Processing and Technology Laboratory and Department of Electrical and Computer Engineering, University of Wisconsin-Madison, Madison, Wisconsin 53706

Grzegorz Sabat and Michael R. Sussman
Department of Biochemistry, University of Wisconsin-Madison, Madison, Wisconsin 53706

Yoshio Nishi
Department of Electrical Engineering, Stanford University, Stanford, California 94305

J. Leon Shohet[a)]
Plasma Processing and Technology Laboratory and Department of Electrical and Computer Engineering, University of Wisconsin-Madison, Madison, Wisconsin 53706

(Received 12 April 2015; accepted 27 August 2015; published 16 September 2015)

A high concentration of radicals is present in many processing plasmas, which affects the processing conditions and the properties of materials exposed to the plasma. Determining the types and concentrations of free radicals present in the plasma is critical in order to determine their effects on the materials being processed. Current methods for detecting free radicals in a plasma require multiple expensive and bulky instruments, complex setups, and often, modifications to the plasma reactor. This work presents a simple technique that detects reactive-oxygen radicals incident on a surface from a plasma. The measurements are made using a fluorophore dye that is commonly used in biological and cellular systems for assay labeling in liquids. Using fluorometric analysis, it was found that the fluorophore reacts with oxygen radicals incident from the plasma, which is indicated by degradation of its fluorescence. As plasma power was increased, the quenching of the fluorescence significantly increased. Both immobilized and nonimmobilized fluorophore dyes were used and the results indicate that both states function effectively under vacuum conditions. The reaction mechanism is very similar to that of the liquid dye. © *2015 American Vacuum Society*.
[http://dx.doi.org/10.1116/1.4930315]

I. INTRODUCTION

Because plasmas are conductive and respond to electric and magnetic fields and can be an efficient source of radiation, they are commonly used for a wide variety of applications. During ionization, a large number of free radicals can also be produced in the plasma depending on the gas composition. These species can significantly affect the application for which the plasma is being used.[1,2] In materials processing, for example, plasma formed from reactive gases, which is likely to contain radicals, is commonly used in various processing steps such as deposition, ashing, and etching.[1,3] Among the plasma species, the radical densities can also be much larger than the density of electrons and ions.[4] These radicals can induce significant chemical and physical modifications to materials under exposure and often enhance or degrade the material properties.[5,6] This is because many free radicals are highly chemically reactive species that can undergo strong chemical reactions with the plasma-exposed material.

Low-k dielectrics, commonly used in the back end of integrated circuits, are particularly susceptible to damage from interactions with radicals. This can lead to methyl depletion, moisture uptake, and surface densification.[5] Consequently, the essential electrical and mechanical characteristics of the dielectric are affected, increasing the dielectric constant, leakage currents, and weakening its structure.[6] It has also been reported that the extent of damage to thin dielectric films is directly related to the densities and types of active species that are present during plasma processing.[6–8] Thus, detecting the types of radicals present in a processing plasma and measuring their effects on materials processing is a subject of high interest.

Oxygen plasma, commonly used in various stages of semiconductor processing, produces species such as singlet oxygen ($^1O_2$) and superoxide anions ($^{\cdot}O_2$–).[9] Although plasma systems are typically pumped down to a low base pressure, traces of moisture, and nitrogen still remain in the vacuum chamber even if the feed gas is not one of the components of ambient air. This can lead to the formation of hydroxyl radicals ($^{\cdot}$HO), nitric oxide (NO), and peroxynitrile anions (ONOO–) as well.[10] A significant amount of all these radicals is present in many processing plasmas.[8]

A. Free-radical detection methods

Reactive species have characteristics that make them difficult to detect, e.g., their very short lifetime along with the presence of other species existing *in situ*, all of which are capable of capturing and/or interacting with the active species. It is therefore essential to utilize metrology that examines the concentration and fluences of these radicals. Several methods have been previously developed to detect radicals in plasmas.[4] However, there are several shortcomings in a

[a)]Electronic mail: shohet@engr.wisc.edu

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number of these experimental methods that hinder investigators from achieving a comprehensive measurement of the effects of radicals on materials. No direct measurements have been made of the flux of each radical species incident on the surface of the material because of the lack of appropriate techniques. The most reliable and commonly used methods to detect radical and other active species in processing plasmas are laser-induced fluorescent spectroscopy and vacuum-ultraviolet absorption spectroscopy.[4] These techniques often require expensive, bulky equipment and often require modifications of the plasma-processing reactor. Moreover, although these methods are useful for free-radical detection and measurements in the bulk plasma, they are not suitable for measurements of the flux of radicals incident on the sample.

B. Fluorophores

An ideal free-radical sensor would be sensitive at low concentrations, radical-selective, usable in vacuum, well characterized, readily available, easy to use without specialized apparatus, and cost effective. Fluorescent dyes offer high sensitivity and selectivity to free radicals and can be analyzed using fluorometry, with the potential to create two-dimensional images of radical concentrations with high spatial resolution.[11]

A fluorophore is a material that can re-emit photons after photon excitation.[12] The fluorophore absorbs photons at a specific wavelength and emits photons at a longer wavelength. The absorbed wavelength depends on the fluorophore structure and its chemical environment, as well as the interaction of the fluorophore molecule in its excited state with surrounding molecules.[13] The wavelength for maximum photon absorption and its resulting emission wavelength is expressed as the ratio of the absorption to the emission wavelengths (e.g., 485/517 nm) and is often used to classify a given fluorophore. The absorption-wavelength spectrum can be very narrow or broadband. The emission spectrum is usually narrower than the excitation spectrum, and it is always of a longer wavelength and correspondingly lower energy. Excitation energies range from ultraviolet through the visible, and emission energies may range from the visible to the near infrared region.[14] Fluorophore dyes typically contain several combined aromatic groups as well as plane or cyclic molecules with several π bonds.[15] These dyes are commonly used to label bioactive reagents (antibodies, peptides, and nucleic acids) and other organic compounds. A wide variety of fluorescent dyes is available including fluorophores that are designed to react with specific kinds of free radicals and/or active species.

Alexa 488 is a highly selective fluorophore and is sensitive to oxygen radicals. It emits a bright green fluorescence (515 nm) when excited at a wavelength of 490 nm.[16] Oxygen radicals react with the dye, degrading it and rendering it unable to fluoresce. This process is called quenching. This is similar to the mechanism by which high-intensity light photobleaches dyes.[17] The number of reactions can be determined by the amount of fluorescent quenching of the dye and can be used to infer the relative concentrations of oxygen free radicals present. Alexa 488 is also thermally stable and the fluorescence lifetime of the dye is temperature independent over the range of temperatures used in this work.[18]

C. Immobilized and nonimmobilized fluorophores

The fluorophores are typically dissolved in an appropriate solvent to a desired concentration before use. Although they are commonly used in liquid form, the dye can also be immobilized on a surface using an appropriate volatile solvent. For many biomedical applications, for example, fluorophores are often immobilized on various surfaces. This immobilization can then be an integral part of many biological assays. For several decades, microspheres with immobilized fluorophores were used to calibrate flow cytometers.[19,20] Recently, there has been a large surge in microarray technology applied to the analysis of gene expression[21–24] and to the study of protein activities[25,26] and tissues.[27] In most cases, the microarray format involves biomolecules labeled with fluorophores and immobilized on dried surfaces. It has been shown that Alexa 488 retains its properties and functionalities when used in an immobilized state for applications in cellular systems.[28]

This technique can be very useful for many plasma applications, especially when used under vacuum, since it prevents the dye from evaporating. In this work, Alexa 488 was immobilized in the bottom of a well of a microtiter plate and exposed to air plasma in order to assess its effectiveness under vacuum conditions. Methanol was used as the drydown solvent. The volume of the dye solution is critical in this case, since a smaller volume will allow rapid evaporation of the volatile solvent leaving the dye immobilized. However, if the solution does not evenly cover the bottom surface of the well, it may result in inconsistent fluorescence readouts, making the fluorometric analysis difficult and inaccurate. In this work, both immobilized and nonimmobilized Alexa were used for comparison.

II. EXPERIMENT

A. Nonimmobilized Alexa

Immediately before use, a working solution of Alexa 488 was prepared by diluting a 1 mg/ml stock solution [initially dissolved in high quality, anhydrous dimethylsulfoxide (DMSO)] to approximately 15-μM (1:100 dilution) with additional DMSO. It was determined that 15-μM of Alexa 488 provided sufficient fluorescence for fluorometric analysis and the quenching of the fluorophore was easily detectable after exposing it to air plasma. DMSO was chosen as a solvent because Alexa is highly soluble in anhydrous DMSO and it has relatively high viscosity and low volatility. Very little loss in volume of the dye solution was observed at pressures down to 10 mTorr.

Five sets of 15-μM, 200 μl Alexa solution were prepared and placed in a 96-well microtiter plate. Each well is 7 mm in diameter and 11.25 mm deep. Five wells were used for each set of samples. Set 1 was not exposed to the plasma and was used to obtain the baseline fluorescence intensity of Alexa 488. The fluorescence of the dye was immediately

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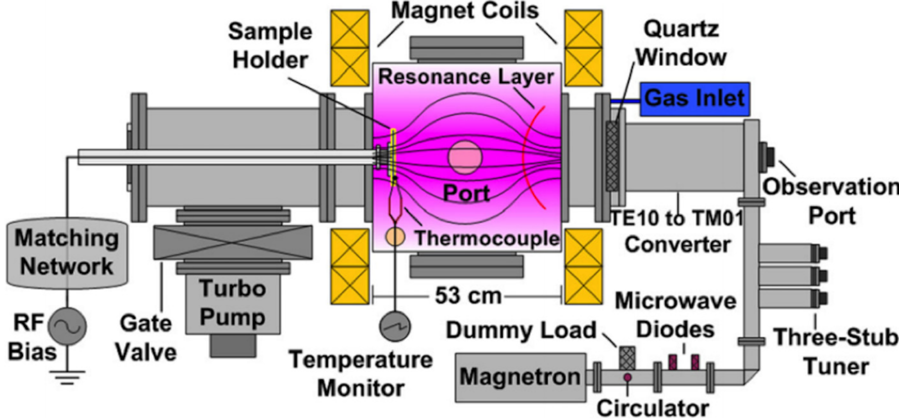

Fig. 1. (Color online) Schematic of the Electron-Cyclotron Plasma Reactor used in this work.

measured after preparation and/or exposure. Set 2 was left uncovered so it was fully exposed to the plasma. Set 3 was covered with a thick piece of silicon and used as a control. The silicon cover was sealed to the wells with Kapton tape to prevent any interaction of particles or radiation from the plasma with the fluorophore. Set 4 was covered with a piece of glass, which was also sealed to the wells with Kapton tape, that minimizes the flux of species from the plasma (ions, electrons, radicals, and neutrals) to reach the Alexa while letting visible-light photons pass through. To investigate the effects of plasma-photon irradiation on the Alexa solution, set 5 was covered with a vacuum ultraviolet (VUV)-grade lithium-fluoride window that allows transmission of VUV photons[28] up to an energy of 11.6 eV. The samples were prepared in a dimly lit room and the well tray was wrapped up with aluminum foil while transporting it to the plasma chamber to minimize exposure to light to minimize the deterioration of the fluorophore molecule.

Plasma exposures were made in an electron-cyclotron-resonance (ECR) plasma reactor operating with a 2.45 GHz microwave power source and 875 G magnetic field as illustrated in Fig. 1.[29] ECR reactors are used in plasma processing and are known for their ability to produce dense plasma.[30] The microtiter plate containing the dye was placed inside the plasma vacuum chamber at the location of the sample holder, which was located approximately 30-cm downstream from the cyclotron-resonant layer, as shown in Fig. 1. No RF bias was applied to the sample holder for these experiments. The fluorescence before and after plasma exposure was measured using a Spectra Fluor Plus Microplate reader fluorometer from TECAN Corporation. The fluorescence intensity is dependent on the gain of the fluorometer, the volume and concentration of the fluorescent dye, and the solvent used to dissolve the dye. For each set of experiments reported in this paper, all of these parameters were kept constant to ensure proper comparison of the fluorescence intensity values.

B. Immobilized Alexa

At low pressures, liquids tend to evaporate quickly so it is difficult to retain the initial amounts of dye solutions at such pressures. Thus, solidified fluorophores that are immobilized on a surface are more useful for vacuum applications. Also, immobilization on a surface will enable the dye to be "painted" on vertically and/or horizontally mounted surfaces to obtain spatial profiles of the radical concentration.

To make an immobilized fluorophore for this work, the stock solution of Alexa 488 (containing 1% DMSO) was dissolved in methanol rather than DMSO to a concentration of 15-μM. Twenty-five microliters of this solution, which completely covered the surface of the bottom of the wells, was added to five wells of the microtiter plate. The dye solution was left undisturbed for about 30 min in ambient room temperature to allow the solvent to evaporate and let the dye settle on the bottom of the wells. The dye was kept in a dark room in order to prevent photobleaching.

The immobilized Alexa was exposed to the ECR air plasma with microwave power ranging from 100 to 1000 W. The pressure and the plasma-exposure time were kept constant at 100 mTorr and 60 s, respectively. Similarly to the nonimmobilized experiments, a control set was utilized in which the immobilized Alexa was completely covered with a sealed silicon cover to prevent any plasma exposure. The fluorometric results for both immobilized and nonimmobilized Alexa are presented and discussed in Sec. III.

III. RESULTS AND DISCUSSION

A. Nonimmobilized Alexa

From Table I, it is seen that the fluorescence of the Alexa 488 dye was quenched by about 50% after 2 min of air-plasma exposure. The quenching can take place from the presence of singlet oxygen, hydroxyl, peroxyl, and other oxygen radicals produced in the plasma.[31] No significant difference in fluorescence was observed between sets 1 and 3. This indicates the Alexa dye remains chemically stable and unmodified under vacuum conditions for a considerable amount of time. By comparing the fluorescence of set 3 (Alexa covered with thick silicon) with that of sets 4 and 5 (Alexa covered with glass and the lithium fluoride window, respectively), it is seen that visible light and UV/VUV radiation from the plasma had little or no effect on the fluorescence of the dye. As a control, the dye samples were also exposed to Argon plasma in order to investigate the

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TABLE I. Measured fluorescence values for nonimmobilized Alexa. Deviations shown are based on an average of 8–10 samples per point.

Set No.	Cover	Treatment	Fluorescence (arb. units)
1	None	No plasma treatment	7398 ± 122
2	None	Air plasma (80 mTorr, 100 W, 120 s)	3898 ± 36
3	Silicon	Air plasma (80 mTorr, 100 W, 120 s)	7406 ± 108
4	Glass	Air plasma (80 mTorr, 100 W, 120 s)	7338 ± 116
5	LiF window	Air plasma (80 mTorr, 100 W, 120 s)	7367 ± 111

influence of ion, electron, and neutral bombardment and any thermal effects on the dye during the plasma exposure. The Argon plasma treatment was done at 150-mTorr pressure and 500 W microwave power for 1 min. Fluorometric measurements after the Argon plasma treatment showed that the fluorophore lost about 8% of its initial fluorescence. When air plasma was used under these conditions, the dye lost over 70% of its fluorescence. The small decrease in fluorescence after the plasma treatment is attributed to a small amount of residual air remaining in the chamber during the plasma treatment. Hence, the change in fluorescence after plasma exposure is primarily from its reaction with oxygen radicals present in the air plasma.

In order to examine how changes in plasma parameters affect the fluorescence of the dye, a concentration of 15-μM Alexa 488 was exposed to air plasma in an ECR reactor for several plasma conditions and exposure times. Figure 2 shows the initial fluorescence response of Alexa 488 dye as a function of exposure time. The pressure and microwave power were kept constant at 100 mTorr and 250 W, respectively, for all the exposures. The control set was always present for each exposure in order to compare the changes in fluorescence of the dye after interacting with the plasma. As can be seen in Figs. 2 and 3, the control set gave repeatable fluorescent intensities for all sets of experiments regardless of the plasma parameters, as expected.

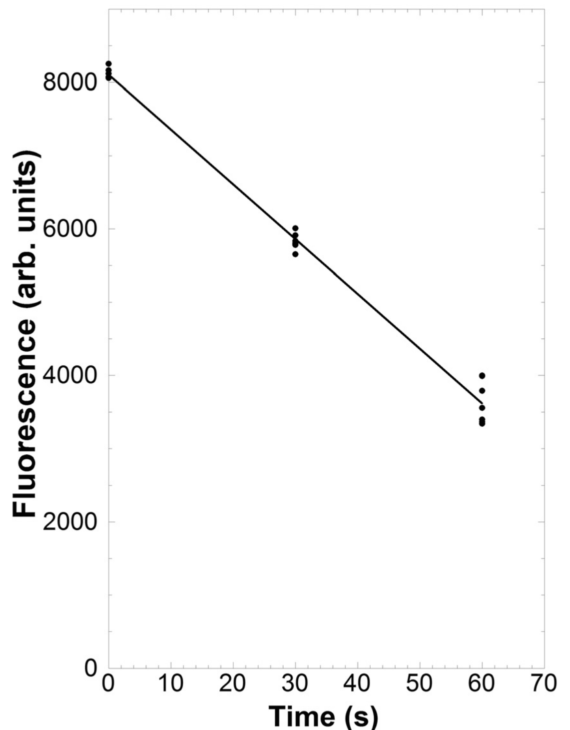

FIG. 2. Fluorescence of non-immobilized Alexa 488 in DMSO as a function of plasma-exposure time.

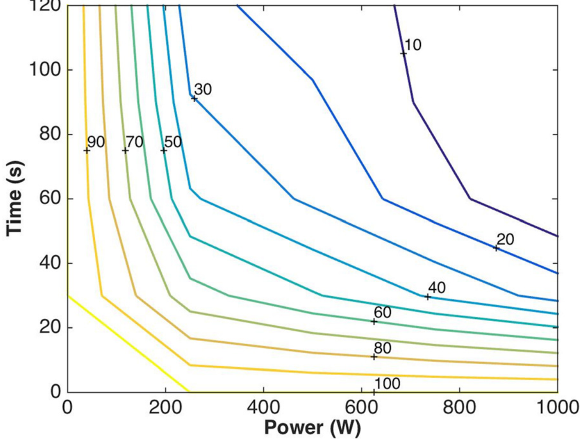

FIG. 3. (Color online) Contour plot of the fluorescence (%) of Alexa at various microwave powers and exposure times.

Figure 3 shows that the initial degradation rate of the fluorescence is inversely proportional to the rf power at the same operating pressure. This is expected because the radical density produced by an ECR plasma increases with microwave power.[32] After the initial drop in fluorescence (as shown in Fig. 2), the dye reached a constant fluorescence value after about 1–2 min of plasma exposure and little quenching was observed for longer exposure times. When a high microwave power (~1000 W) was used to produce the plasma, the fluorescence of Alexa was completely eliminated after about 1 min of plasma exposure. Thus, the steady-state value of the fluorescence is also inversely proportional to the rf power.

We conclude that the steady state fluorescence is produced by those fluorophore molecules near the bottom of the microtiter plate wells because the free radicals do not have enough energy to penetrate to the bottom of the wells. As the rf power is increased, the radicals have more energy and can then penetrate to the bottom of the wells at the highest power eventually causing complete quenching of the fluorophore.

B. Immobilized Alexa

For comparison, immobilized Alexa was also exposed to air plasma under similar conditions. After plasma exposure, fluorometric analysis was performed and the results were compared to the fluorometric data from nonimmobilized Alexa. The results are shown in Table II and Fig. 4. The

TABLE II. Fluorescence of immobilized Alexa 488.

Set No.	Cover	Treatment	Fluorescence (arb. units)
1	No	No plasma treatment	3945 ± 245
2	Silicon	Air plasma (100 mTorr, 1000 W, 480 s)	3888 ± 322
3	No	Air plasma (100 mTorr, 1000 W, 60 s)	26 ± 1

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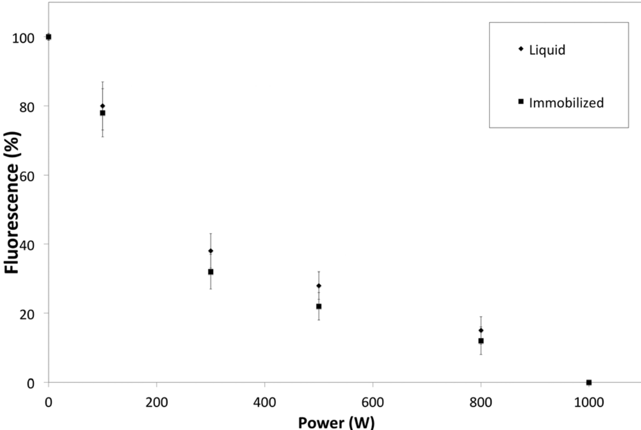

FIG. 4. Fluorescence quenching of liquid and dry Alexa as a function of microwave power.

fluorometric data obtained from the control set (set No. 2) indicate that the fluorescence of the dye does not degrade until it is exposed to oxygen radicals. Figure 4 shows the fluorescence response of both liquid and dry 15-μM Alexa as a function of the microwave power. The plot shows that the quenching of the fluorescence of immobilized Alexa replicates the trend obtained with the nonimmobilized Alexa in DMSO solution.

IV. SUMMARY AND CONCLUSIONS

Alexa 488 fluorophores were employed in a plasma-processing system to detect the presence of oxygen radicals at the location of the sample holder. The fluorescent dye reacts selectively with oxygen radicals and the fluorescence of the dye is degraded after the interaction. In order to investigate the effectiveness of Alexa 488 under vacuum conditions, the dye was dissolved in DMSO solvent and exposed to air plasma in the wells of a microtiter plate. Using fluorometric analysis, quenching of the fluorescence of the dye was observed after plasma exposure. This indicates the fluorophore has reacted with oxygen radicals from the plasma. The extent of the fluorescent degradation of Alexa is directly related to the oxygen radical density.[33–36] Experimental results show that the fluorescence of Alexa reaches a saturation point, which suggests that the quenching of the dye no longer increases. The steady-state quenching occurred earlier as microwave power was increased indicating higher radical energies in the plasma. The steady-state quenching level is attributed to the fact that the free radicals cannot penetrate to the bottom of the wells unless they have enough energy. Both nonimmobilized and immobilized fluorophores show similar results. As has been shown, by varying the plasma exposure time, the level of quenching as a function of radical fluence can be elucidated. Quantitative measurements of radical flux can be determined using a known source of free radicals. Since many types of fluorophores exist that selectively react with individual species, a wide range of free radicals can be detected and their relative concentrations can be measured using this technique.

ACKNOWLEDGMENTS

This work has been supported by the Semiconductor Research Corporation under Contract No. 2008-KJ-1871, the National Science Foundation under Grant No. CBET-1066231 and Grant No. MCB 0929395, and under DOE Contract No. DEFG02-88-ER13938.

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