Abstract—Measurement of temperatures with high spatial resolution in fluid is a challenging topic with many useful applications. This work presents a novel technique for extracting the temperature a few nanometers away from the surface with extremely high spatial resolution via the use of a covalently attached fluorescent dye, 5(6) carboxyfluorescein. The emission characteristics of the dye are highly temperature dependent, which allows the construction of a calibration curve that can be used to measure unknown temperatures directly at surfaces with spatial resolution only limited by the size of the employed fluorophores.

Index Terms—fluorescence, thermometry, covalent, temperature

I. INTRODUCTION

The measurement of temperature at the nanoscale with high spatial resolution remains a challenging topic. In many applications, from integrated circuits to diagnosis of damaged blood vessels to lab-on-a-chip applications, an accurate, fast measure of temperature is critical [1-7]. Particularly with lab-on-a-chip applications, enzyme activated reactions [8], polymerase chain reaction (PCR) amplification of DNA [9-10], and chemical and biomolecular sensors require precise control and measurement of temperature at the micro and nanoscales. Many methods have been developed thus far to extract the temperature in the bulk fluid above these lab-on-a-chip devices, varying from lithographically fabricated or vapor-deposited thermocouple detectors [11], which suffer from low spatial resolution due to geometry, to the use of temperature sensitive fluorophores in fluid [12]. However, these methods typically do not measure the temperature directly at the interface between the fluid of interest and the active surface of the chip, which can vary drastically from both the temperature in bulk fluid far above the device and the temperature at thermocouples far away from the active region of the devices.

II. FLUOROPHORE ATTACHMENT

To address this problem, a technique is demonstrated for the precise measurement of temperatures within a few nanometers of a surface with high spatial resolution. The technique uses covalently attached temperature sensitive fluorophores that are anchored to a silicon dioxide surface. First, a SAM of 3-aminopropyl-dimethylethoxysilane is vapor deposited on a silicon dioxide surface as has been demonstrated previously [13]. Next, 5 (6) carboxyfluorescein (chemical structure shown in Figure 1a), a temperature and pH dependent fluorophore is anchored to the silane layer. The activated succinimidyl ester of the molecule, which links to the amine groups of the silane, is circled in red. The resulting stack, which results in a fluorophore located about 5 nanometers from the surface, is shown in Figure 1b.

Manuscript received April 09, 2010. Work supported in part by NIH (R01CA20003).
B. Reddy, Jr. is with the Department of Electrical and Computer Engineering, University of Urbana-Champaign, Urbana IL 61801 USA (e-mail: reddyjr1@illinois.edu).
B. R. Dorvel is with the Department of Biophysics and Computational Biology, University of Urbana-Champaign, Urbana IL 61801 USA (e-mail: bdorvel2@illinois.edu).
O.H. Elibol is with Intel Corporation, Santa Clara CA 95054 USA (e-mail: oguz.h.elibol@intel.com).
P. Nair is with the Department of Electrical and Computer Engineering, Purdue University, West Lafayette IN 47907 USA (e-mail: pnair@purdue.edu).
M.A. Alam is with the Department of Electrical and Computer Engineering, Purdue University, West Lafayette IN 47907 USA (e-mail: alam@purdue.edu).
D. E. Bergstrom is with the Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette IN 47907 USA (e-mail: bergstrom@purdue.edu).
R. Bashir is with the Department of Electrical and Computer Engineering and the Department of Bioengineering, University of Urbana-Champaign, Urbana IL 61801 USA (e-mail: rbashir@illinois.edu).
Fig. 1. (a) Structure of the 5 (6) carboxyfluorescein used in the experiments. The dashed red circle highlights the ester that hooks to the silane groups on the oxide surface. (b) Schematic demonstrating the concept of the temperature measurement. On the left, when the sample is at room temperature, the emitted fluorescence is at a normal level. On the right, when the sample is heated, the measured fluorescence decreases.

III. CALIBRATION CURVE

To determine the correlation between temperature and the excited fluorescence of the covalently attached fluorophores, a calibration was needed. The covalent attachment was performed on the silicon dioxide gate dielectric of FET dual heater-sensor hybrids which have been demonstrated previously [14]. An open microfluidic well made from PDMS was placed over the chip, and 10x tris-borate-EDTA buffer (Sigma) was dropped into the well. Next, a cover glass was placed over the chip and the chip was transferred to a heating chuck under a fluorescent microscope (Nikon Eclipse 600 with a Penguin 600 CL cooled CCD camera). Fluorescent intensity readings were taken from regions directly over the devices at five temperatures ranging from 25°C to 65°C (Figure 2) to obtain the calibration curve. A line was fitted to the data to determine the relationship between temperature and the ratio of the observed fluorescence at elevated temperature to that at room temperature (25°C).

IV. TEMPERATURE MEASUREMENT

Next, the devices were heated using an AC microwave method that has been shown previously (Figure 3a) [14]. The temperature of the devices was extracted using the calibration curve. From this, a relationship between the applied AC heating voltage and temperature could be determined (Figure 3b). Also, a heat map demonstrating the high spatial resolution of the technique was generated, for an applied heating AC voltage of 40 Vrms (Figure 3c). Since the fluorophores themselves have sizes on the order of nanometers, the practical spatial resolution of the technique is limited mostly by the optics of the camera. Furthermore, because the fluorophores are located around 5 nm off the surface, these results truly describe the temperature at the immediate vicinity of the active surface with the bulk fluid above.

V. CONCLUSION

In this work, we have demonstrated that a covalently attached fluorophore can be used to monitor the temperature within a few nanometers of oxide surfaces with high spatial resolution. These results can be used in a variety of applications, from PCR to chemical and biosensors.
Fig. 3. (a) Schematic demonstrating the AC technique, adapted from [14]. The source and drain of the sensors are shorted together, and an AC voltage is applied between this node and the bulk (backgate) of the device. (b) Data showing the correlation between the applied AC voltage and the measured temperature on the surface of the sensor-heaters. (c) Raw fluorescent image of a device heated with an applied AC voltage of 40 V, with the corresponding heat map demonstrating the high spatial resolution of the technique.