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Microwave-induced thermogenetic activation of single cells

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Exposure to a microwave field is shown to enable thermogenetic activation of individual cells in a culture of cell expressing thermosensitive ion channels. Integration of a microwave transmission line with an optical fiber and a diamond quantum thermometer has been shown to allow thermogenetic single-cell activation to be combined with accurate local online temperature measurements based on an optical detection of electron spin resonance in nitrogen–vacancy centers in diamond. © *2015 AIP Publishing LLC*. [http://dx.doi.org/10.1063/1.4917538]

New technologies enabling a high-precision control over the electrical activity of specific cells in a living organism offer unique opportunities for the functional analysis of complex biological systems, including brain and its functions. Optogenetics is revolutionizing neuroscience through the use of genetically encoded light-sensitive ion channels,[1–5] which enable a spatially precise selective modulation of specific cells within complex distributed networks of neurons, thus offering unique tools for studying the mechanisms whereby the dynamics of these networks controls cognitive responses, memory, learning, and behavior. As a promising alternative, thermogenetics uses thermosensitive ion channels[6–10] to drive the cell activity by temperature variations. With a vast family of thermosensitive ion channels available,[11–14] broad ranges of working temperatures and activation thresholds can be covered, allowing neurons expressing these channels to be switched from silent to robustly active mode by a slight change in temperature. However, despite all these breakthroughs, applications of thermogenetics in neuroscience studies are limited, primarily to experiments with fruit flies,[6,10] by the lack of tools enabling a precisely controlled, well-localized control of temperature inside living systems, which would prevent heating that would be incompatible with the general physiology of the organism and help avoid increased background activity of cells, such as a spontaneous firing of neurons.

In this work, we demonstrate a thermogenetic activation of individual cells in a cell culture using a microwave field. Thermal effects induced by microwave fields in biological systems have been a subject of extensive studies (see, e.g., Ref. 15 for a review), motivated, to a large extent, by public health considerations. In a standard scheme of a microwave experiment, a living matter is exposed to a wide-field microwave radiation, delivered by an external microwave source, which increases the temperature within the entire microwave-irradiated region. Since the wavelength of microwave radiation is orders of magnitude larger than the typical cell size, modulation of individual cells is not feasible in this experimental scheme.

In the experiments presented in this paper, a microwave field is delivered to a region of interest through a miniature microwave transmission line. This approach will be shown to enable a well-controlled local heating and thermogenetic activation of individual cells. Moreover, a microwave transmission line integrated with an optical fiber and a diamond quantum thermometer will be used to demonstrate thermogenetic single-cell activation combined with accurate local online temperature measurements based on an optical detection of electron spin resonance in nitrogen–vacancy (NV) centers in diamond.

Experiments were performed (Fig. 1) on a culture of Human Embryonic Kidney 293 (HEK-293) cells grown in a Petri dish. The HEK-293 cells were transfected with vectors expressing Green-GECO calcium indicator[16] and rattlesnake TRPA1 channels, known to be responsible for remote thermosensation by rattlesnake *Crotalus atrox*.[8] To this end, HEK-293 cells (ATCC) were seeded into 35 mm glass bottom dishes (MatTek) and cultured in DMEM with 10% FCS (PAA Laboratories) at 37 °C in a 5% CO_2 atmosphere.[17] After 24 h cells were transfected by a mixture of 1 ng DNA (or 0.65 ng DNA of each vector for co-transfection) and 3 μl

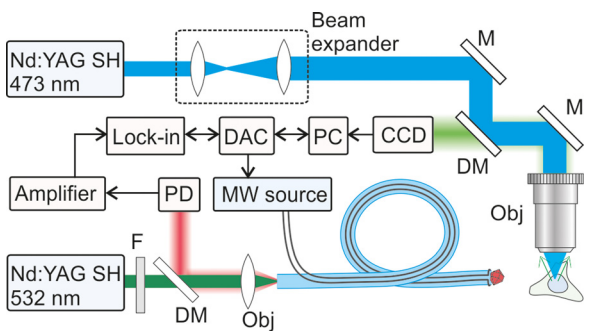

FIG. 1. Diagram of the experimental setup: Nd: YAG SH, Nd: YAG lasers with a second-harmonic output; Lock-in, lock-in amplifier; PD, photodetector; DAC, digital-to-analog converter; Obj, microscope objectives; M, totally reflecting mirror; DM, dichroic mirror; CCD, charge-coupled device camera; and GM, galvanoscanned mirror.

(6 μl for co-transfection) X-treme GENE 9 transfection reagent per one dish. After 8–10 h, cell medium was replaced by fresh medium. After 36–48 h of transfection, HEK-293 cells were incubated for 2 h in MEM without bicarbonate supplemented with 20 mM of HEPES-NaOH, pH 7.4, at the room temperature. A Petri dish with the cell culture was placed on a translation stage on a high-precision adjustable microscope table. Cell imaging was performed using a 10× microscope objective and a CCD camera. The cells were irradiated with a continuous-wave 473-nm diode-laser output, which provided optical excitation of Green-GECO.

A fiber probe used in our experiments integrates[18,19] a microwave transmission line, an optical fiber, and an NV-diamond quantum sensor. The microwave field is delivered through a two-wire transmission line, which consists of a pair of copper wires 50 μm in diameter each, running along the optical fiber and short-circuited with an open-ring section, winding around the fiber tip (Fig. 1). The microwave transmission line is precisely positioned in the cell culture using a homebuilt high-precision mechanical manipulator. With the electric and magnetic fields rapidly falling off with the distance from the open-ring antenna, the microwave-induced heating of the cell culture gives rise to a rapidly decaying temperature gradient, which confines thermogenetic activation to a group of a few cells adjacent to the open-ring microwave antenna (Fig. 1).

Online local temperature measurements are performed by using the temperature-dependent frequency shift of optically detected magnetic resonance,[20] which is induced by coupling the microwave field, delivered through the microwave transmission line, to NV centers in diamond on the tip of the fiber probe. In the absence of external magnetic fields, the $m_s = 0$ and $m_s = \pm 1$ sublevels of the ground-state triplet of NV centers are split by $\Omega_s \approx 2.87$ GHz (Fig. 2(a)). The 532-nm optical field spin-polarizes NV centers, accumulating them in the $m_s = 0$ state through spin-selective decay paths.[21–23] This laser radiation couples the ^3A ground electronic state to the ^3E excited state, giving rise to photoluminescence (PL), featuring a characteristic zero-phonon line, which is observed at approximately 637 nm at room temperature against a broad phonon-sideband line, stretching down to 800 nm. Photoluminescence emitted by laser-initialized NV centers within the 630–800-nm wavelength range is collected by the same optical fiber,[18,19] and is transmitted through this fiber to the detection system, consisting of a silicon photodiode, a low-noise preamplifier, and a lock-in amplifier (Fig. 1).

For NV centers in the $m_s = \pm 1$ state, the PL yield is lower than that typical of NV centers in the $m_s = 0$ state, because a substantial fraction of the $m_s = \pm 1$ excited-state population is transferred to the $m_s = 0$ ground level via a metastable singlet state (the ^1A state in Fig. 2(a)), which does not contribute to the 630–800-nm PL band. The intensity of the PL signal, I_{PL}, therefore decreases when a microwave field delivered by the transmission line integrated into our fiber probe is tuned to the zero-field splitting frequency Ω_s, transferring population from the $m_s = 0$ state to the $m_s = \pm 1$ sublevels (Fig. 2(a)). A local strain in the diamond lattice removes the degeneracy of the $m_s = \pm 1$ sublevels, giving rise to two well-resolved features (maxima or minima,

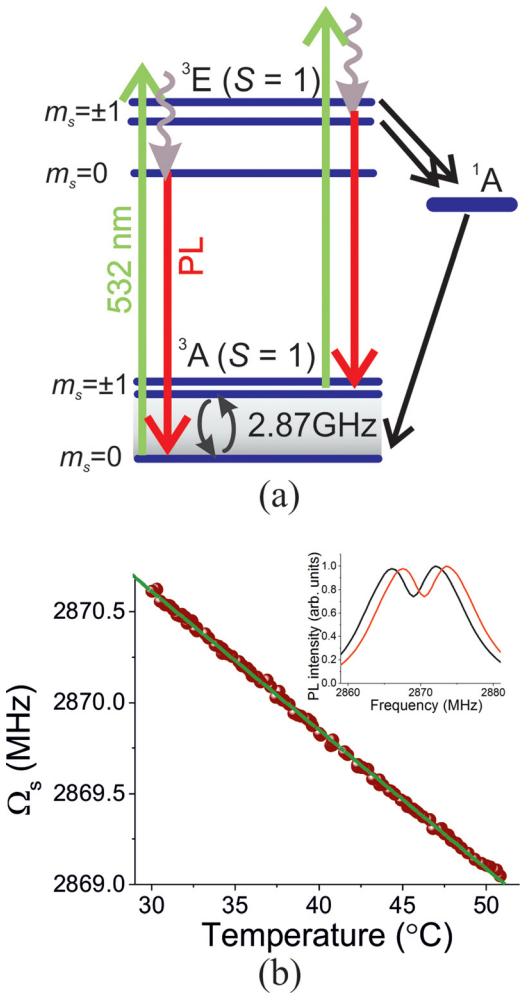

FIG. 2. Diagrams of optical excitation and photoluminescence readout (left) and energy levels involved in the optical detection of magnetic resonance in NV centers in diamond. (b) The magnetic-resonance frequency Ω_s as a function of the temperature T inside a thermostat: (dots) experiment and (solid line) the best linear fit. The inset shows zero-external-magnetic-field profiles $I_{PL}(\Omega)$ measured with $T \approx 30$ °C (red line) and 50 °C (black line).

depending on the detection technique) in the PL intensity I_{PL} measured as a function of the microwave frequency Ω (the inset in Fig. 2(b)). As the temperature of diamond increases, this profile of the zero-external-magnetic-field resonance is shifted[20] toward lower microwave frequencies, enabling temperature measurements with a high spatial resolution. For the highest sensitivity and highest speed of local temperature measurements in a cell culture, frequency-modulated microwave spin excitation in NV centers was combined with properly optimized differential lock-in detection.[19,24]

In a calibration experiment, the fiber probe was placed inside a thermostat with a precisely controlled temperature along with a thermocouple, providing an accuracy of temperature measurements higher than 0.1 °C. Figure 2(b) displays the magnetic-resonance frequency Ω_s measured as a function of the temperature inside the thermostat according to thermocouple readings. As can be seen from this plot, a linear function with a slope $d\Omega_s/dT \approx -76.3 \pm 0.2$ kHz/K (solid line in Fig. 2(b)) provides an ideal fit for this dependence within the entire temperature range of interest, viz., from 30 °C to 50 °C, offering a convenient calibration for temperature measurements using our fiber-optic probe with NV diamond.

In experiments with a culture of HEK-293 cells expressing the rattlesnake TRPA1 channel and Green-GECO calcium indicator, we analyze a thermal response of a group of seven cells (labeled with arrows in the image in Fig. 3) located within a distance of 120 μm from the open-ring microwave antenna on the tip of the fiber probe, with the nearest cell (cell *1* in Fig. 3) lying at a distance of about 60 μm from this antenna. As a part of such analysis, we take images of the studied group of cells with a speed of 0.5 frames per second and measure the intensity of the fluorescence response from each cell in this group as a function of time. The output power of the microwave source is increased by 5 dBm at the moments of time $t \approx 580$ s, 870 s, and 1200 s, shown by vertical dashed lines in Fig. 4. No cell activation is observed for $t < 580$ s in Fig. 4, when the temperatures of all the cells are below the activation threshold of TRPA1 channels, $T_a \approx 27$ °C.

Within the time interval from 580 to 870 s, the microwave power is adjusted in such a way as to heat cell *1* to a temperature above T_a, but to keep the temperature of the other, more distant cells below this threshold. At $t \approx 610$ s, as can be seen from Fig. 4, the Green-GECO fluorescence response from cell *1* starts to display well-resolved spikes (black line in Fig. 4(a)), indicating the flow of Ca^{2+} through the cell membrane. The other cells, as can also be seen from Fig. 4(a), including those lying within a distance of about 90 μm from the microwave antenna (cells *2* and *3* in Fig. 4(a)), show no sign of activation.

At $t \approx 870$ s, the microwave power is increased by another 5 dBm, giving rise to a further increase in the temperature of the cells. Within the time interval from 870 to 1200 s, all the cells within a distance of about 120 μm from the open-ring microwave antenna are seen to display spikes in their fluorescence (Figs. 4(a) and 4(b)), signaling the activation of TRPA1 channels on their membranes. Finally, as the microwave power is further increased at $t \approx 1200$ s, leading to a further heating of the cell culture, all the cells in the studied group become activated, displaying regular spikes in their fluorescence response. Experiments on a reference culture of HEK-293 cells, where the cells were transfected with vectors expressing Green-GECO calcium indicator without TRPA1 channels, did not show cell activation at any time (the red line in Fig. 4(b)) within the entire range of microwave powers used in our experiments.

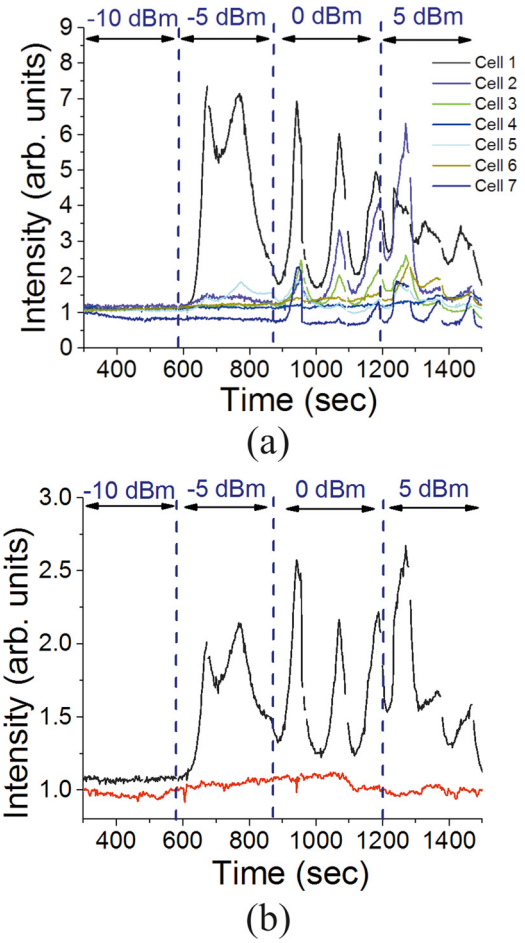

FIG. 4. Intensity of fluorescence from the Green-GECO indicator measured as a function of time (a) for individual cells and (b) for the entire group of seven cells in the cell culture exposed to microwave radiation, delivered through the microwave transmission line. The microwave power is increased by 5 dBm at the moments of time $t \approx 580$ s, 870 s, and 1200 s, shown by the vertical dashed lines. The fluorescence response from the Green-GECO indicator in a reference culture of HEK-293 cells, where the cells were transfected with vectors expressing Green-GECO calcium indicator, but not TRPA1 channels, are shown by the red line.

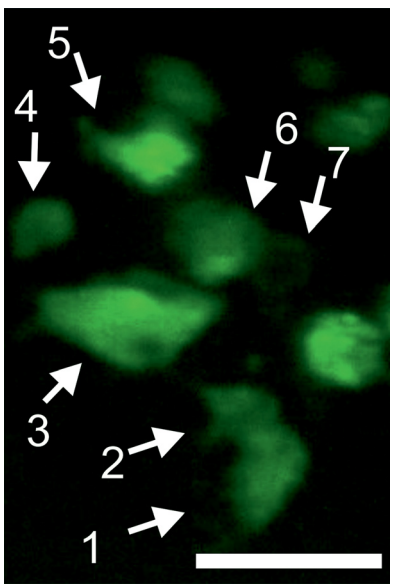

FIG. 3. An image of a group of seven HEK-293 cells expressing the rattlesnake TRPA1 channel and Green-GECO calcium indicator in a cell culture exposed to microwave radiation, delivered through the microwave transmission line. The scale bar is 50 μm.

To summarize, we have demonstrated a thermogenetic activation of individual cells with a microwave field delivered to the cells of interest through a miniature microwave transmission line. Integration of a microwave transmission line with an optical fiber and a diamond quantum thermometer has been shown to allow thermogenetic single-cell activation to be combined with accurate local online temperature measurements based on an optical detection of electron spin resonance in NV centers in diamond.

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