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ABSTRACT

Intracellular temperature is a crucial parameter that influences metabolic processes, enzymatic activity, and cellular signaling. Advances in nanoscopic thermometry using nitrogen-vacancy (NV) centers in diamond provide unprecedented precision in mapping temperature variations within living cells. NV centers, atomic-scale defects in the diamond lattice, exhibit quantum properties sensitive to temperature, enabling non-invasive and high-resolution thermal measurements. These properties, combined with the biocompatibility and stability of nanodiamonds, make them ideal for probing cellular thermodynamics in real time. By integrating NV-based thermometry with multi-electrode array (MEA) techniques, it is possible to correlate intracellular temperature variations with changes in cellular metabolism and electrophysiological activity. This combination offers unique insights into how metabolic processes influence cellular function, particularly under varying physiological and pathological conditions. For example, mapping temperature heterogeneity within cellular microenvironments can shed light on mitochondrial thermogenesis or metabolic alterations in disease states. This approach also holds promise for studying the role of intracellular temperature in the development and progression of neurodegenerative diseases, where metabolic dysfunction is a peculiar indicator. Another intriguing application could involve the functionalization of nanodiamonds to target specific organelles, enabling even more precise investigations of localized thermal dynamics in the future. For now, current advancements in NV-based thermometry already demonstrate its robustness as a tool for exploring the dynamic interplay between intracellular temperature and metabolism. These insights provide a foundation for advancing our understanding of cellular energetics and for developing innovative approaches in diagnostics and therapeutics.

Keywords: quantum sensing, intracellular thermometry, Nitrogen-Vacancy center, ODMR, nanodiamonds

INTRODUCTION

The nitrogen-vacancy (NV) center in diamond has emerged as a versatile quantum sensor for nanoscale measurements of temperature, magnetic fields , and electric fields[1]. This atomic-scale defect in the diamond lattice consists of a substitutional nitrogen atom adjacent to a lattice vacancy. The electronic ground state of the NV center is a spin triplet ($|m_s=0\rangle$, $|m_s=\pm1\rangle$) with a zero-field splitting (ZFS) of $D_{gs}\sim2.87$ GHz[2,3]. Changes in environmental conditions, such as temperature, induce shifts in the ZFS, making NV centers exceptionally suited for precise thermometry[4,5,6].

The key to utilizing NV centers lies in the optically detected magnetic resonance (ODMR) technique. Green laser illumination initializes the system into the $|m_s=0\rangle$ state, while microwave radiation induces transitions between $|m_s=0\rangle$ and $|m_s=\pm1\rangle$[7]. The NV center's photoluminescence is spin-dependent[8]: the $|m_s=0\rangle$ state exhibits higher red fluorescence compared to the $|m_s=\pm1\rangle$ states, due to the presence of an intersystem crossing (ISC). This ISC pathway leads to non-radiative relaxation when the system starts from $|m_s=\pm1\rangle$, causing a decrease in fluorescence. By monitoring shifts in the ODMR spectrum, temperature changes can be accurately detected[9,10].

Intracellular temperature plays a fundamental role in regulating metabolic activity, ion channel dynamics, and cellular signaling[11,12]. While NV-based thermometry has previously been used to study thermal dynamics[13] and multi-electrode arrays (MEAs) have independently been employed for electrophysiological measurements[14], their simultaneous application has been a missing link. The simultaneous measurement of intracellular temperature and electrophysiological activity, such as neuronal firing, could provide valuable insights into the interplay between thermal dynamics and cellular function, paving the way for deeper investigations of how temperature variations influence metabolic and electrophysiological processes under both physiological and pathological conditions[15].

EXPERIMENTAL SECTION

Correlation between temperature variations and neurons firing [15]

In this recent study NV-based nanodiamond thermometry and multi-electrode arrays (MEAs) were exploited to investigate the relationship between intracellular temperature variations and neuronal activity in cultured hippocampal neurons[15]. While these two methodologies were employed separately, their results were integrated to provide a comprehensive understanding of the interplay between thermal dynamics and electrophysiological processes.

For temperature measurements, neurons were incubated with nanodiamonds containing NV centers, which were internalized into the cells. Intracellular temperature changes were monitored by detecting shifts in the zero-field splitting (ZFS) of the NV centers' ODMR spectrum under three experimental conditions (Figure 1a): spontaneous neuronal firing in control conditions, potentiated activity induced by picrotoxin (a GABAA receptor antagonist), and silenced activity achieved through tetrodotoxin (TTX) and cadmium chloride. These measurements revealed temperature increases of approximately 1°C during potentiated activity and decreases of around 0.5°C during silencing.

Separately, MEAs were employed to record neuronal firing patterns under the same experimental conditions (Figure 1b). This allowed the characterization of changes in firing rates associated with the temperature variations observed in the thermometric experiments. By combining these complementary datasets, the study demonstrates how neuronal activity correlates with intracellular temperature dynamics, shedding light on the metabolic and electrophysiological interplay in neurons.

Careful calibration ensured the viability of the cells and minimized artifacts, confirming that the observed temperature changes were intrinsic to neuronal activity (Figure 1b). This dual-method approach highlights the potential for future experiments combining simultaneous measurements of thermometry and electrophysiology.

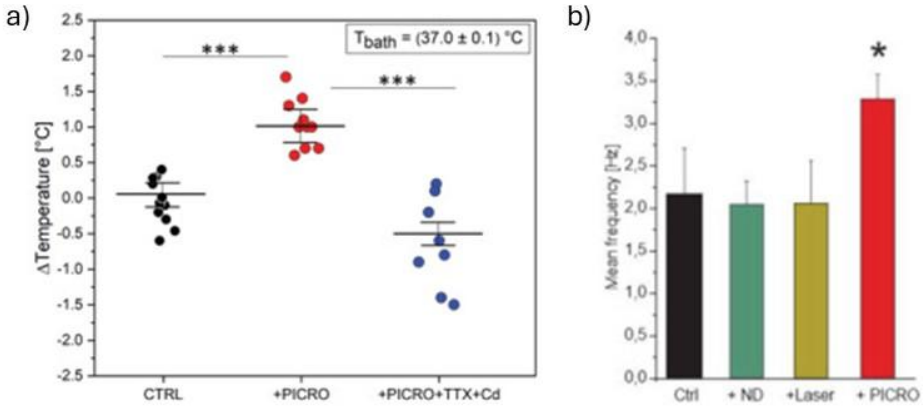

Figure 1. Intracellular temperature dynamics and neuronal activity. (a) Temperature changes measured via nanodiamonds with NV centers: ~1°C increase during potentiated activity (picrotoxin) and ~0.5°C decrease during silenced activity (TTX and cadmium chloride) compared to controls. (b) MEA recordings show corresponding changes in neuronal firing rates under the different conditions.

Synchronized measurements

The findings from this study underscore the critical need to measure intracellular temperature variations and neuronal firing simultaneously to establish a direct correlation between these phenomena. While the separate application of NV^- based thermometry and MEAs has provided valuable insights into the relationship between thermal dynamics and electrophysiological activity, the temporal decoupling of the measurements limits the ability to definitively link specific temperature changes to precise firing events.

Temperature variations in neurons are closely tied to processes such as ion channel activity, action potential generation, and metabolic shifts, all of which occur on rapid timescales. Without real-time synchronization, the observed thermal changes may only provide an averaged or indirect view of the dynamic processes underlying neuronal excitability. Simultaneous measurements would allow for a more precise temporal mapping of temperature fluctuations relative to neuronal firing patterns, providing clearer insights into the causal relationships between these processes.

Moreover, concurrent thermometry and electrophysiological measurements could shed light on unexplored mechanisms, such as localized thermogenesis during action potential propagation or the thermal effects of neurotransmitter release. This approach would also enable the investigation of pathological conditions, such as neurodegenerative diseases, where both metabolic dysfunction and altered firing patterns play pivotal roles.

By integrating NV-based thermometry and MEA technology into a unified platform, future research could achieve real-time, high-resolution mapping of the interplay between intracellular temperature and neuronal activity, significantly advancing our understanding of neuronal function and dysfunction.

Confocal setup

The experimental setup utilized a confocal microscope integrated with components for optically detected magnetic resonance (ODMR) measurements, optimized for high-resolution imaging and precise optical and microwave-based measurements. A confocal configuration allows for signal collection exclusively from a single focal plane, reducing out-of-focus noise and enhancing spatial resolution[16] (Figure 2a). This capability is critical for nanoscale measurements and three-dimensional imaging of intracellular structures. The system includes a laser source for excitation, a high numerical aperture (NA) objective lens for precise focusing and efficient signal collection, and a photodetector for sensitive fluorescence detection. Emitted photoluminescence is filtered to remove scattered light and ensure that only the desired signal reaches the detector. For biological measurements, the confocal microscope is integrated with an inverted optical one. An incubator placed on the top of an XYZ stage maintains a stable temperature of the environment, ensuring cell viability during measurements. To enable ODMR, the setup incorporates a microwave generator coupled with a broadband antenna. The microwave generator produces signals that are amplified and delivered to the sample via the antenna, providing a strong and homogeneous electromagnetic field (Figure 2b). This configuration facilitates precise control of the spin states of NV centers, enabling temperature measurements with nanoscale resolution. The combination of confocal microscopy with microwave and incubation systems creates a powerful platform for biological experiments, allowing simultaneous control over environmental conditions and precise optical and microwave-based measurements at the cellular and subcellular levels.

The confocal setup described above can be combined with a microelectrode array (MEA) system to extend its capabilities to electrophysiological measurements. MEAs are versatile tools consisting of arrays of electrodes that allow extracellular recording of neuronal activity across multiple sites simultaneously. By positioning neurons or cell cultures directly onto the MEA, it is possible to capture electrophysiological signals such as spikes or local field potentials with high spatial and temporal resolution. This combined platform enables simultaneous measurements of intracellular temperature, using the NV-based thermometry setup, and neuronal activity recorded via the MEA. Such an integrated approach facilitates the correlation of thermal dynamics with neuronal firing patterns, addressing critical questions about the interplay between temperature and cellular function.

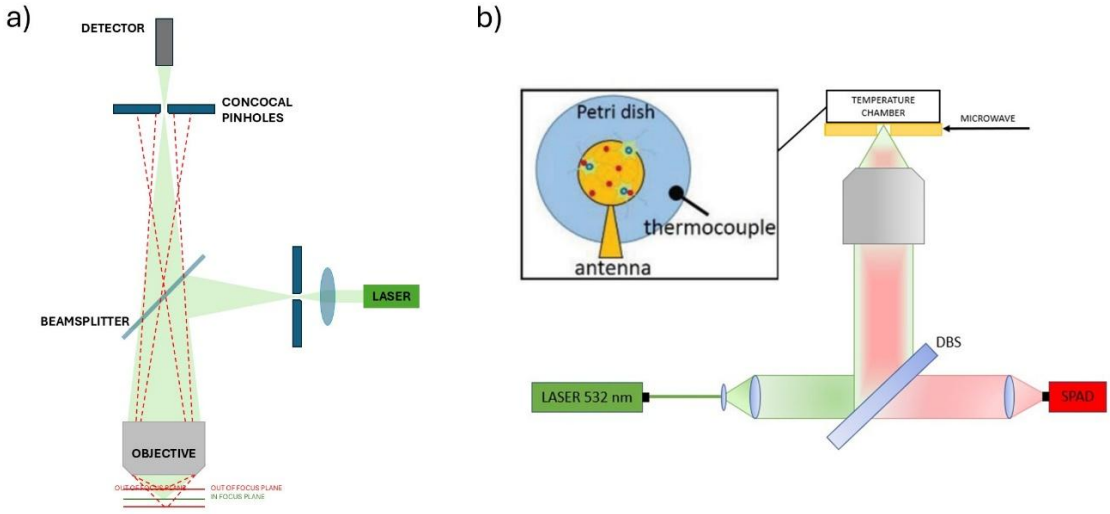

Figure 2. Confocal microscopy principles and setup. (a) Schematic of confocal microscopy: out-of-focus light is excluded, allowing high-resolution imaging of specific focal planes. (b) Diagram of the custom confocal microscope designed for biological measurements in this study.

Characterization of the system

The first step toward synchronizing electrical and optical measurements involved integrating the MEA system into the setup and thoroughly characterizing it. To this end, a set of nanodiamonds was employed to monitor operator-induced temperature variations using ODMR techniques.

The sample preparation consisted of depositing nanodiamonds into a MEA chip and adding 1 mL of distilled water. By exploiting the MEA system's ability to modulate temperature, controlled temperature variations were induced and monitored using a thermocouple submerged in the water. Simultaneously, the resonance value of the nanodiamonds was tracked, and the coupling constant γ_T , that links temperature variations to ZFS shifts, was calculated using the following equation.

$$\Delta_{ZFS} = \gamma_T \cdot \Delta_T \rightarrow \gamma_T = \frac{\Delta_{ZFS}}{\Delta_T} \quad (1)$$

Measurements were performed on a set of 5 nanodiamonds, with the results summarized in Table 1 and visualized in Figure 3. The data were acquired under 100uW green laser radiation (532 nm). From these data, an average coupling constant of γ_T = (81 ± 2) kHz/K was obtained, which aligns well with values reported in the literature[5,10]

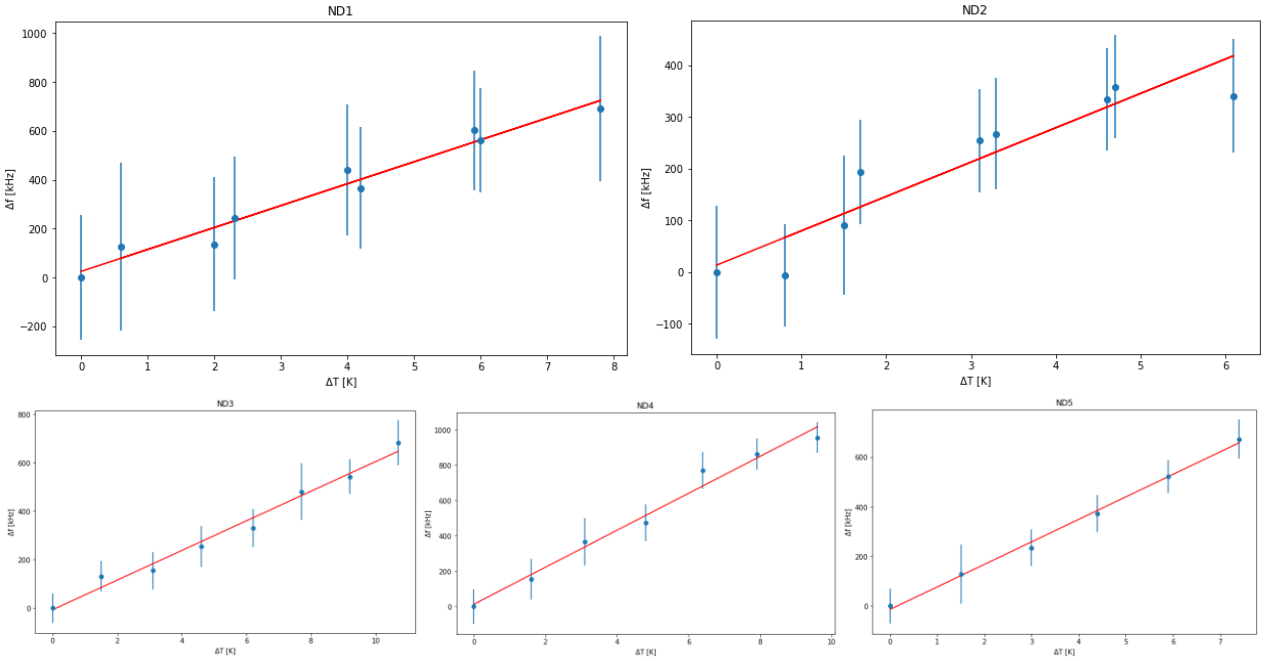

Figure 3: Calibration curves obtained from 5 different nanodiamonds

Nd	$\gamma_T\ [kHz\ K^{-1}]$
1	90 ± 6
2	66 ± 9
3	61 ± 3
4	104 ± 7
5	91 ± 3

Table 1: Coupling constant obtained from 5 different nanodiamonds

These results demonstrate the feasibility of integrating ODMR-based thermometry with electrical measurements conducted via MEAs. This marks a significant step toward achieving synchronized optical and electrical measurements in future experiments.

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