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Photoacoustic micropipette

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Glass micropipette electrodes are commonly used to target neurons, either in vitro or in vivo. They acquire electrophysiological recordings for the purpose of developing a further understanding of the behavior of neurons at the single cell and network levels. The success rate of acquiring adequate recordings during these procedures, however, is largely limited. Here, we demonstrate how a photoacoustic micropipette (PMP) electrode is capable of providing real-time photoacoustic feedback, useful in navigation towards intended targets. The PMP is fabricated from standard pulled borosilicate glass micropipettes, coated with aluminum. Light introduced into the wall of the micropipette, parallel to the axis, travels along the entire length of the device before exiting the tip, where it can induce the photoacoustic effect. © 2018 Author(s). All article content, except where otherwise noted, is licensed under a Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/). https://doi.org/10.1063/1.5052162

Glass micropipette electrodes are a powerful tool for providing electrophysiological recordings of neuronal activity within both in vitro and in vivo biological systems. This fundamental information has enabled a better understanding of neuronal activity, indicative of cell and network function. However, inherent system complexity leads to difficulties accurately acquiring cellular recordings, mainly dependent on the skill of the user. These recordings are often done with either a sharp or patch micropipette. A typical sharp micropipette will have a tip diameter that approaches or exceeds the limit of resolution of most optical microscopes. Thus, this technique, although performed under high optical magnification, is carried out without knowledge of the exact location of the micropipette tip, relative to the neuron. This makes it difficult to target the neuron and acquire recordings with high efficiency. Patch micropipettes, used to perform traditional patch clamping, have a tip diameter larger than sharp micropipettes, on the scale of a few microns. The larger tip size allows for the patch micropipette to be more readily and accurately positioned near the intended target. The lack of real-time feedback regarding neuronal movement often results in a poor gigaohm seal. Several attempts have been made to increase the successful acquisition of intracellular recordings, yet these techniques still (i) suffer from low success rates, (ii) depend strongly on the skill of the electrophysiologist, (iii) rely heavily on cell labeling, or (iv) are limited by the penetration depth of light. Non-imaging techniques such as monitoring changes in impedance at the pipette tip have been implemented to increase the rate of successful recordings. The highest acquisition efficiencies for these non-imaging techniques reach a modest 51% for in vivo models. Imaging techniques have also been implemented, such as oblique epi-illumination, shadow-patching, or targeting of fluorescently labeled neurons under one-photon and two-photon microscopy. The most successful of these imaging techniques employs the combination of two-photon microscopy and fluorescence. This approach has a success rate of roughly 50% for in vivo recordings. Imaging based approaches have led to increased rates of successful recordings but are not without drawbacks. The reliance on optical microscopy ensures that these techniques will not be effective for locating neurons that are beyond the penetration depth of light. Moreover, utilizing fluorescence requires expensive reagents and specialized infrastructure. To address these issues, this paper introduces a type of targeting approach that may enable increased rates of successful neuronal recordings, without the need for exogenous contrast agents. Reported here is a technique that incorporates a photoacoustic feedback system into traditional micropipette electrodes.

Among recent scientific advancements in biomedical engineering, the photoacoustic effect has shown great medical promise. In brief, this phenomenon is defined by ultrasound waves produced by small thermal expansions, caused by optical absorption. This enables detection of particular absorbers through the use of specific wavelengths that match their peak optical absorbance. Recent studies have taken advantage of these absorption peaks to create image reconstructions of cellular and tissue structures using photoacoustic microscopy. Typically, this imaging technique is performed by concentrating light to a small spot size, which can then be used to generate the photoacoustic effect at specific spatial locations. A different method of concentrating light is through the use of an optical waveguide, which tapers down to a small diameter. Light is able to travel down the length of the waveguide. Immediately upon exiting the tip, the light is the same diameter as the waveguide, before increasing in diameter. Traditional glass micropipette electrodes, due to their material properties (i.e., borosilicate glass and quartz glass), can be used as hollow optical waveguides. With minor adjustments, light coupled to the glass wall can be efficiently guided down the length (5.8 cm) of the micropipette electrode and out of the tip. The properties of the sound generated by the light traveling along the...
micropipette can reveal information regarding the presence of a particular absorber along the path of the micropipette. This enables a photoacoustic feedback system that can be used to move the micropipette toward a particular absorber, creating an integrated photoacoustic micropipette (PMP).

A tunable LS-2134-LT40 Nd:YAG/Ti:Sapphire nanosecond pulsed laser (Symphotic TII Corporation) operated at a 460 nm wavelength is used as the excitation source to induce photoacoustic signals. The laser provides an excitation light with a full width at half maximum (FWH M) of 12–15 ns at a pulse repetition rate of 10 Hz. Two methods have been utilized to control light through the PMP. In the first method, an achromatic doublet lens (ACN127-050-A, Thorlabs) is used to focus an \( \sim 140 \mu J \) light pulse onto the back end of the PMP. This method of coupling requires the pulsed light to travel by total internal reflection along the entire length of the PMP before exiting the tip. A second method of introducing light through the PMP is by way of an optical fiber (FG105ACA, Thorlabs). The optical fiber is fed through the hollow center of the PMP and emits an \( \sim 50 \mu J \) light pulse near the tip. Pulsed light exiting the PMP tip was measured to have a mean value of \( \sim 40 \text{nJ} \) per pulse. Differences in energy levels between the two methods are due to variations in coupling efficiency. Photoacoustic signals generated by the pulsed light can be detected near the tip or at the back end of the PMP using different configurations. Given the large differences in distance that the signal would have to travel to be detected at these two spatial locations, the central frequencies of the transducers were chosen to maximize both the resolution and sensitivity. In configuration 1 (i.e., fixed), a 50 MHz transducer with an element diameter of 6 mm and a \(-6 \text{dB} \) fractional bandwidth of 82\% (V214-BB-RM, Olympus) has a fixed position near the PMP tip, below the target. The short distance between the two methods are transducer and the target enables the use of a high frequency transducer for improved axial resolution. Signals detected by this transducer are sent through an independent amplification system consisting of two amplifiers in series [ZFL-500LN-BNC(+), Mini-Circuits]. In configuration 2 (i.e., coupled), a 10 MHz transducer with an element diameter of 2 mm and a \(-6 \text{dB} \) fractional bandwidth of 54\% (XMS-310-B, Olympus) is placed in contact with the back end of the PMP. Due to the attenuation of high-frequency ultrasound over the length of the 5.8 cm distance between the coupled transducer and the target, a transducer with a relatively low central frequency was utilized. Signals detected by this transducer are sent through an independent amplification system consisting of an ultrasound pulser/receiver (5077PR, Olympus Inc.). These signals are amplified using a 59 dB gain and filtered with a 1 MHz high pass filter. Data acquisition of the signals from the two independent amplification systems is acquired with a multipurpose reconfigurable oscilloscope (NI PXIe-5170R, National Instruments Corporation). To allow for custom triggering and synchronization of the system, the oscilloscope has integrated programmable function input/output (PFI) lines controlled by a built in field programmable gate array (FPGA).

A borosilicate capillary tube with an outer diameter of 1 mm and an inner diameter of 0.75 mm is pulled using a P-87 micropipette puller (Sutter Instruments). The average inner diameter of the PMP tips is measured to be approximately 3.5 \( \mu \text{m} \). In order to facilitate the propagation of light along the length of the micropipette, a 200 nm thick layer of aluminum is applied to the outer surface. The thickness of the coating on the micropipette is estimated using a reference piezo sensor during the deposition process. The film deposition is visualized using scanning electron microscopy (SEM), as shown in Fig. 1. The results indicate a smooth and continuous coating on the outer surface of the PMP, which is necessary in order to prevent the formation of pinholes. Pinhole formation may allow the light to escape prior to reaching the tip.

The beam shape of the light exiting the tip of the PMP depends on several factors. One strong determining factor of the light exiting the tip of the PMP is the angle of incidence of the light coupled to the device. Large angles of incidence result in poor transmission of light along the entire length. In order to ensure maximum light transmission, the light is coupled to the PMP with a near zero angle of incidence. A second method of maximizing light transmission is through the use of an optical fiber fed into the PMP. Our research has shown that both methods can yield similar beam profiles if the coupling efficiencies are taken into account. Shown in Fig. 2(b) is a representative beam profile, as investigated using the experimental setup depicted in Fig. 2(c). In brief, the tip of the PMP is placed into a 30 \( \mu \text{M} \) droplet of fluorescein solution. The resulting fluorescence is captured using a CCD microscope camera, equipped with a FITC filter. The beam profile indicates that the fluence of the light decreases with the increasing distance from the tip. Thus, as the PMP tip approaches a target, the resulting photoacoustic signal is likely to increase due to the increased fluence.

Figure 3(a) is a schematic representation of the experimental setup used to evaluate the capacity of the PMP to induce the photoacoustic effect. A 254 \( \mu \text{m} \) diameter carbon fiber rod is submerged in distilled water within a small Petri dish, under which the fixed transducer is positioned. The position of the Petri dish is controlled by a three-axis motorized stage (PatchStar, Scientifica). The stage moves horizontally in a direction perpendicular to the orientation of the

![FIG. 1. SEM image of the PMP tip.](image-url)
target in 2.5 \( \mu \text{m} \) steps. The vertical position of the PMP, above the Petri dish, allows the target to cross paths with the PMP during horizontal movement. The laser exiting the PMP induces a photoacoustic signal only when the target and the PMP are aligned [Fig. 3(b)]. The generated photoacoustic signal is detected by the fixed transducer positioned directly below the Petri dish. The stage is then used to move the Petri dish vertically toward the PMP. As the photoacoustic target approaches or recedes from the PMP tip, a corresponding increased or decreased photoacoustic signal is generated, as shown in Fig. 3(c). Initially, the stage is moved vertically towards the PMP in 50 \( \mu \text{m} \) steps until a noticeable increase in photoacoustic signal is detected, at which point the step size is decreased to 10 \( \mu \text{m} \). After multiple 10 \( \mu \text{m} \) steps, the step size is further decreased to 1 \( \mu \text{m} \) steps. The embedded graph in Fig. 3(c) highlights the increase in the photoacoustic signal after each 1 \( \mu \text{m} \) step. These results identify the ability of the PMP to vertically approach targets through guided signal intensity.

Further studies to evaluate the capacity of the PMP to detect targets of different sizes are performed using an experimental setup resembling Fig. 3(a). In these experiments, the laser is coupled to the PMP by way of an optical fiber. The PMP position is controlled using a micromanipulator (PatchStar, Scientifica), and the spatial position of the targets is fixed. Finally, the coupled transducer is positioned against the back end of the PMP to detect the photoacoustic signals that travel up the glass walls. A 254 \( \mu \text{m} \) diameter carbon fiber rod and a 7.2 \( \mu \text{m} \) diameter carbon fiber thread are used as line targets. Each of the targets is independently submerged in distilled water within small Petri dishes. The micromanipulator is used to move the vertically oriented PMP in a horizontal direction in micron-sized steps. At each micron-sized step, the laser is fired down the PMP and 1000 ns of data from the resulting photoacoustic signal is acquired and filtered. An analytical signal is created by combining the data, \( s(t) \), with its own Hilbert Transform, \( H[s(t)] \). The magnitude of the analytical signal is subsequently calculated in order to create a complex envelope, \( s_e(t) \), of the original data, such that \( s_e(t) = \sqrt{s^2(t) + H^2[s(t)]} \). This envelope is then normalized and converted into a series of pixel values. Each series of pixel values is then plotted as an independent column in the image reconstruction. To remove the background noise from the overall image, the average value across each of the rows was calculated and subtracted from each pixel value within that row.

Shown in Figs. 4(a) and 4(b) are photoacoustic image reconstructions of the 254 \( \mu \text{m} \) line target, using the fixed transducer and coupled transducer, respectively. The maximum photoacoustic signal generated at each horizontal step when scanning the 254 \( \mu \text{m} \) line target is shown in Figs. 4(c) and 4(d), for the fixed transducer and coupled transducers, respectively. Using a Gaussian fit, the measured transverse FWHM is 154 \( \mu \text{m} \) for the fixed transducer and 162 \( \mu \text{m} \) for the coupled transducer. This indicates that the resulting
The photoacoustic image is clearly indicative of the size of the target, suggesting that the PMP can be navigated to be directly above the target. Maximum signals generated along the vertical direction for the 254 \( \mu \text{m} \) line target are displayed in Figs. 4(e) and 4(f). Measuring the FWHM using a Gaussian fit and a speed of sound of 1498 m/s resulted in a value of 41 \( \mu \text{m} \) for the fixed transducer and 243 \( \mu \text{m} \) for the coupled transducer. Differences in the horizontal FWHMs measured in Figs. 4(c) and 4(d), and the actual size of 254 \( \mu \text{m} \), are likely due to the large diameter and curvature of the target in comparison with the tip diameter. Thus, horizontal scanning across the target results in a changing vertical distance between the target surface and the PMP tip. As shown previously in Figs. 2(b) and 3(c), large differences in distance can result in large differences in fluence and subsequently in photoacoustic signal intensity. Thus, measuring the signal along the vertical direction becomes dependent on generating the photoacoustic effect roughly 254 \( \mu \text{m} \) from the PMP tip. Furthermore, the measurement made by the coupled transducer in the vertical direction, as shown in Fig. 4(f), may be more representative of the axial resolution. Although the 10 MHz transducer would typically provide better spatial resolution, the large attenuation of any high frequency signals traveling through the PMP may ultimately decrease the axial resolution.

Further experiments were performed on 7.2 \( \mu \text{m} \) line targets. Shown in Figs. 5(a) and 5(b) are photoacoustic image reconstructions of the 7.2 \( \mu \text{m} \) line target, using the fixed transducer and coupled transducer, respectively. The reconstruction of this line target using the coupled transducer [Fig. 5(b)] was processed using a 2-D Gaussian smoothing kernel, prior to background removal. This is done to further amplify the presence of the 7.2 \( \mu \text{m} \) line target in the reconstruction. The maximum photoacoustic signal generated at each horizontal step when scanning the 7.2 \( \mu \text{m} \) line target is shown in Figs. 5(c) and 5(d), for the fixed transducer and coupled transducer, respectively. Using a Gaussian fit, the measured transverse FWHM is 32 \( \mu \text{m} \) for the fixed transducer and 58 \( \mu \text{m} \) for the coupled transducer. These results identify the ability of the PMP to horizontally locate targets of varying sizes, down to 7.2 \( \mu \text{m} \). Maximum signals generated along the vertical direction for the 7.2 \( \mu \text{m} \) line target are displayed in Figs. 5(e) and 5(f), for the fixed transducer and coupled transducer, respectively. Measuring the FWHM using a Gaussian fit and a speed of sound of 1498 m/s resulted in a value of 57 \( \mu \text{m} \) for the fixed transducer and 265 \( \mu \text{m} \) for the coupled transducer. This difference in the size measured between the fixed and coupled transducer is likely a result of alterations to the ultrasound during transmission through the glass of the PMP. Differences in the horizontal FWHMs measured in Figs. 5(c) and 5(d), and the actual size of 7.2 \( \mu \text{m} \), are likely due to factors within the experimental setup. Scans of the target were performed horizontally across 5 mm and lowered by micron sized steps. This process was repeated until the tip was broken, since a slight tilt of even a half of a degree along the scan direction can result in a height difference of tens of microns across the scan length, from one end to the other. This would suggest that the PMP may have been tens of microns away from the target in both Figs. 5(a) and 5(b). Decreasing the scan length may allow the PMP tip to
gain better proximity to the target and provide improved horizontal resolution.

In this work, we produced PMPs using traditional micro-pipette electrodes as hollow optical waveguides. The PMPs are coated with a continuous smooth layer of aluminum, allowing for controlled light deposition down to a few microns in size. Pulsed light at a wavelength of 460 nm induces a photoacoustic signal from line targets as small as 7.2 μm. Here, we demonstrate that the overall photoacoustic signal increases as the distance between the target and the PMP tip decreases. Here, we show that the induced photoacoustic signals provide information that can reliably be used to move the pipette tip towards the intended target using either a 50 MHz fixed transducer or a 10 MHz coupled transducer. The increased axial resolution of high frequency transducers may make these fixed transducers ideal for in vitro applications, where the detector can be placed directly underneath the sample. Lower frequency coupled transducers, however, may be best suited for in vivo applications where their capacity to detect minimally attenuated signals will prove to be more effective in deep tissue applications.

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