OPTOACOUSTIC IMAGING

Maps of neuronal activity across the mouse brain

High-speed optoacoustic tomography can monitor the neural activity of a whole mouse brain, by using a genetically encoded calcium sensor originally developed for fluorescence microscopy.

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To be able to understand how the brain functions in health and disease, it is necessary to map the neuronal circuitry that gives rise to physiological processes, behaviour and disease states. Neuronal circuitry is a dynamic network with ever-changing connections, in which neuronal activity occurs over a broad range of temporal and spatial scales. A major challenge in brain imaging is to non-invasively record dynamic changes in neuronal structures, as well as neuronal activity, at the cellular level across a large area, and at great depth, in living animals — with the ultimate goal of doing the same in humans. However, the ability to non-invasively monitor neuronal activity with cellular resolution in deep brain regions is hindered by the scattering of light by tissue.

Existing high-resolution light-microscopy modalities, such as two-photon microscopy, combined with ultrasensitive sensors of neuronal activity (such as the GCaMP family of genetically encoded calcium indicators), allows for chronic optical recordings from large populations of neurons in awake and behaving animals. These techniques permit the functional visualization of single neurons at depths of 1 mm at most. And although one-photon miniature fibre optic probes permit the visualization of single neurons in deeper regions, these probes have limited depths of field (few hundreds of μm from the tip of the probe) and fields of view (<1 mm^2 areas)[1].

An emerging approach for deep-brain imaging is optoacoustic (OA) imaging, which uses laser pulses to generate ultrasound waves. Ultrasound scattering by tissues is two-to-three orders of magnitude weaker than optical scattering. In contrast to light microscopy, OA has enabled high-resolution 3D imaging of biological samples at depths that are unreachable via light microscopy. For example, OA computed tomography can image haemodynamic responses of the whole mouse brain through the intact skull[2]. Therefore, OA imaging has the potential to facilitate the visualization of both normal and pathologic activities of neurons with high speed, high spatial resolution and deep penetration. Also, OA imaging has the potential to be correlated with fluorescence imaging, as any molecule that can absorb a photon and then release excited-state energy (partly) via thermal relaxation will generate OA signals. The

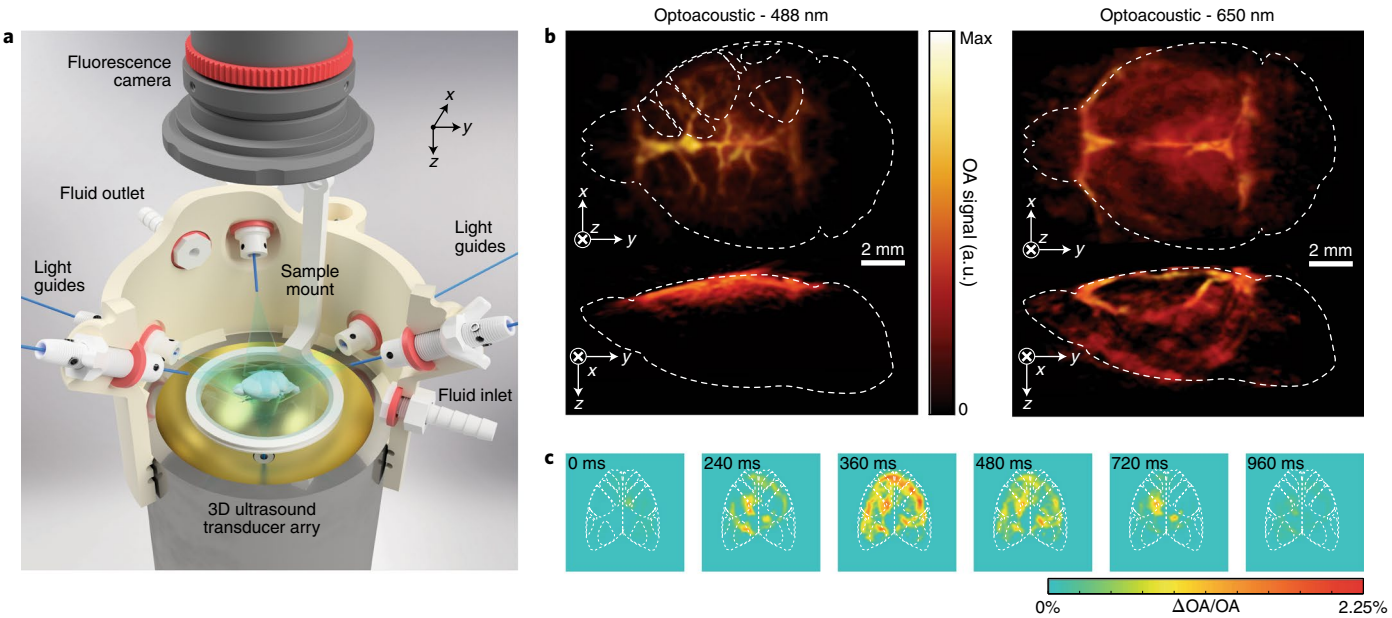

Fig. 1 | Functional optoacoustic imaging of a whole mouse brain. a, The imaging setup is designed to achieve the uniform illumination of a specimen. Seven optical fibres surround and illuminate the sample, which is held in a chamber that allows for continuous perfusion with artificial cerebrospinal fluid. The ultrasound transducer that detects sound waves is underneath the sample, whilst a camera fitted above the sample enables fluorescence recording. **b**, In vivo OA images from a mouse brain expressing the genetically encoded calcium indicator GCaMP, acquired at 488 nm from approximately 1–2 mm deep into the cortex (left), and at 650 nm (a wavelength at which haemoglobin absorption is reduced) from a depth of 7 mm (right). **c**, Relative increase in OA signal for a brain slice at approximately 1 mm in depth in GCaMP6f-expressing live mice at different time points following electrical stimulation of the mouse's hind paw. Figure adapted from ref. [3], Springer Nature Ltd.

scalability of OA imaging also provides the opportunity to link neurophysiological signalling at multiple length scales through multiple optical-absorption contrast agents. By combining a customized functional OA tomography technique with the use of GCaMP, Shy Shoham, Daniel Razansky and colleagues now report in *Nature Biomedical Engineering* high-speed volumetric calcium-dependent OA contrast in the mouse brain, with a volumetric field of view of approximately 2 cm^3 and with 150 μm spatial resolution, covering about one million individual voxels at a temporal resolution of 100 Hz (ref. [3]).

The geometrical arrangement of components in optoacoustic setups is evolving, and is a crucial aspect for obtaining an optimal and homogeneous excitation as well as for enabling sample readouts[4]. Building on their previous work[5], Razansky and colleagues arranged an array of optical fibres around the biological specimen, to provide uniform illumination while delivering light at multiple wavelengths without having to modify the alignment of the fibres (Fig. 1a). A semi-spherical acoustic transducer was positioned underneath the sample and, with 512 elements and wideband detection, it increased the spatial resolution to 150 μm. Above the sample, a camera was used to record fluorescence either in parallel or in series with acoustic detection (this was used to validate the OA-imaging methodology). To enable functional imaging, the authors chose GCaMP6f, which has been widely used for multiphoton imaging in vivo. GCaMP6f is based on a circularly permutated green fluorescence protein, coupling calcium-binding-induced conformational changes to variations in fluorescence emission, and is suitable for OA imaging owing to its differential OA response in the presence and absence of calcium.

Razansky and co-authors first demonstrated brain-wide calcium imaging in an excised GCaMP6f-expressing mouse brain. To prepare the brain, they removed the blood vessels and placed it in a sample holder designed for continuous perfusion of cerebrospinal fluid (Fig. 1a). Next, they recorded a drug-induced brain-wide calcium wave via functional OA. Injection of pentylenetetrazol (a non-competitive antagonist for GABA receptors) induced localized calcium release, which subsequently propagated into calcium waves that could be observed throughout the brain. The authors observed anatomic details of the brain, and showed that the temporal resolution of the response of GCaMP6f was similar to what is normally recorded optically. Fluorescence imaging at superficial layers of the brain, acquired alongside OA imaging, correlated well with it.

Importantly, Razansky and colleagues used OA imaging to non-invasively record, through skin and skull, sensory-evoked calcium activity up to 2 mm into the cortex in vivo (Fig. 1b), despite strong haemoglobin absorption that overlapped with the GCaMP excitation wavelength (hind-paw electric stimuli were used to induce calcium release in the somatosensory cortex). As expected, the OA signal showed bilateral activations, which followed the fluorescence signal and matched the expected dynamics of GCaMP. High-speed volumetric recordings enabled the visualization of the time-dependent evolution of calcium waves (Fig. 1c) at different depths within the cortex, which could ultimately lead to a better understanding of how stimuli-evoked responses travel and are interconnected in spatially distant brain regions. The OA-signal transients were stronger than the fluorescence response, and the contrast provided by the calcium sensor was larger than that of the haemodynamic background.

OA imaging with the use of genetically encoded reporters is, therefore, suitable for observing functional dynamics in the rodent brain. The approach might lead to the routine application of optoacoustics for the direct imaging of neuronal activity in highly vascularized and light-scattering mammalian brains. However, the application of existing ultrasensitive fluorescent sensors, including GCaMP and neurotransmitter sensors (such as iGluSnFR; ref. [6]) or neuromodulator sensors (such as dLight1; ref. [7]), has limited the depth of penetration for OA imaging in mouse brains, owing to high haemoglobin absorption below 650 nm. For deep-tissue OA imaging, fluorescent probes with absorption and emission spectra spanning the near-infrared window (650–900 nm) are highly desired. Such sensors would make it possible to combine the examination of individual neurons by optical microscopy with the examination of macroscopic brain structure and function through OA imaging. Besides genetically encoded probes, a long list of small molecules and nanoparticles are also available as a rich resource of reporter molecules for OA imaging[8,9].

With rigorous development, both in OA setups and in suitable contrast probes, OA imaging may continue to bridge the gap between microscopic and macroscopic scales for the investigation of brain function in rodent models. And with appropriate contrast agents, it may one day be possible for OA imaging to be used for the clinical diagnosis of defective neuronal signalling deep in brain tissue. ❐

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References

1. Vincent, P. et al. *EMBO Rep.* **11**, 1154–1161 (2006).
2. Yao, J. & Wang, L. V. *Curr. Opin. Chem. Biol.* **45**, 104–112
3. Gottschalk, S. et al. *Nat. Biomed. Eng.* https://doi.org/10.1038/s41551-019-0372-9 (2019).
4. Wang, L. V. & Yao, J. *Nat. Methods* **13**, 627–638 (2016).
5. Deán-Ben, X. L. et al. *Light Sci. Appl.* **5**, e16201 (2016).
6. Marvin, J. S. et al. *Nat. Methods* **10**, 162–170 (2013).
7. Patriarchi, T. et al. *Science* **360**, eaat4422 (2018).
8. Miao, Q. & Pu, K. *Bioconjug. Chem.* **27**, 2808–2823 (2016).
9. Knox, H. J. & Chan, J. *Acc. Chem. Res.* **51**, 2897–2905 (2018).