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Dual-channel high-speed functional photoacoustic microscopy with ultra-wide field of view

Van Tu Nguyen¹, Carlos Taboada^{1,2,3}, Jesse Delia³, Tri Vu¹, Luca Menozzi¹, Soon-Woo Cho¹, Jing Li¹, Nishad Jayasundara⁴, Anthony DiSpirito¹ and Junjie Yao¹

Abstract

Photoacoustic microscopy (PAM) systems often face challenges in simultaneously achieving high speed, high resolution, high sensitivity, and a large field of view (FOV). To address this challenge, we have developed dual-channel PAM (DC-PAM) that can expand the FOV without compromising the imaging speed, detection sensitivity, or spatial resolution. DC-PAM has two identical, independent channels of laser excitation and acoustic detection. It exploits two facets of a single hexagon scanner to concurrently steer the dual excitation laser beams and the resultant acoustic waves. DC-PAM achieves an ultra-wide FOV of $22.5 \times 24 \text{ mm}^2$ with a total functional imaging time of $\sim 15 \text{ s}$. Proof-of-concept experiments were conducted using DC-PAM on freely-swimming zebrafish, hypoxia-challenged mice, and sleeping glassfrogs, all of which benefit from the large FOV and high imaging speed to track the dynamic and physiological processes at the whole-organ or whole-body level. These applications demonstrate the potential of DC-PAM for a wide range of biological studies.

Introduction

Over the past decade, advancements in optical imaging technologies have transformed life sciences. High-resolution optical imaging provides fine structural details and physiological activities in tissues^{1–4}. However, achieving high-resolution imaging often comes at the expense of a limited field of view (FOV) or imaging speed⁵. For dynamic imaging applications, such as imaging freely moving organisms, a wide FOV is highly desirable. As an optical imaging technology, photoacoustic microscopy (PAM) enables volumetric imaging by detecting acoustic signals generated through the photoacoustic effect^{6–8}. More importantly, PAM offers functional⁹ and molecular imaging¹⁰ of a large range of endogenous and exogenous contrasts, such as measuring oxygenation^{11,12}, blood flow^{13,14}, and oxygen metabolism^{15–17}.

However, like other optical imaging techniques, PAM faces the technical challenge of simultaneously achieving a

high imaging speed, large FOV, high spatial resolution, and high detection sensitivity¹⁸. Traditional PAM systems rely on slow motorized scanning^{19,20}, but recent advancements in high-speed PAM have introduced faster scanning mechanisms, such as piezo scanners²¹, slider crank scanners^{22,23}, galvo scanners^{24–27}, micro-electromechanical systems (MEMS) scanners^{8,28–31}, and polygon scanners^{32,33}. Among them, the polygon scanner stands out by offering high-speed, repeated line scanning across a large range³⁴. It holds an advantage over galvo and MEMS scanners, as its scanning range is independent of the scanning speed^{35–37}. However, the ultrasound detector in high-speed PAM often limits the FOV or compromises detection sensitivity. These limitations hinder the large-scale imaging tasks, such as whole-body imaging and simultaneous imaging of multiple organisms.

To enhance the performance of high-speed PAM, we introduce a dual-channel PAM (DC-PAM) system equipped with two independent imaging channels. A single water-immersible hexagon scanner is used to steer two laser beams and their resultant PA signals at the same time. This unique design is enabled by the symmetric geometry of the hexagon scanner. Two facets of the hexagon are used simultaneously, resulting in a line scan

Correspondence: Carlos Taboada (carlos.a.taboada@vanderbilt.edu) or Junjie Yao (junjie.yao@duke.edu)

¹Department of Biomedical Engineering, Duke University, Durham, NC 27708, USA

²Biology Department, Duke University, Durham, NC 27708, USA

Full list of author information is available at the end of the article

These authors contributed equally: Van Tu Nguyen, Carlos Taboada.

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that is twice as wide as that in previous polygon-based PAM systems^{38–40}. We have demonstrated that DC-PAM can achieve an ultra-wide FOV of $22.5 \times 24 \text{ mm}^2$, with a total imaging time of 15 seconds. Using dual-wavelength excitation, we have validated that DC-PAM can simultaneously image the hemodynamic responses in two mice, one under normoxia and the other under hypoxia. Furthermore, we have shown that DC-PAM can map the functional dynamics of glassfrog transparency at the whole-body level and track the group behavior of freely-swimming zebrafish.

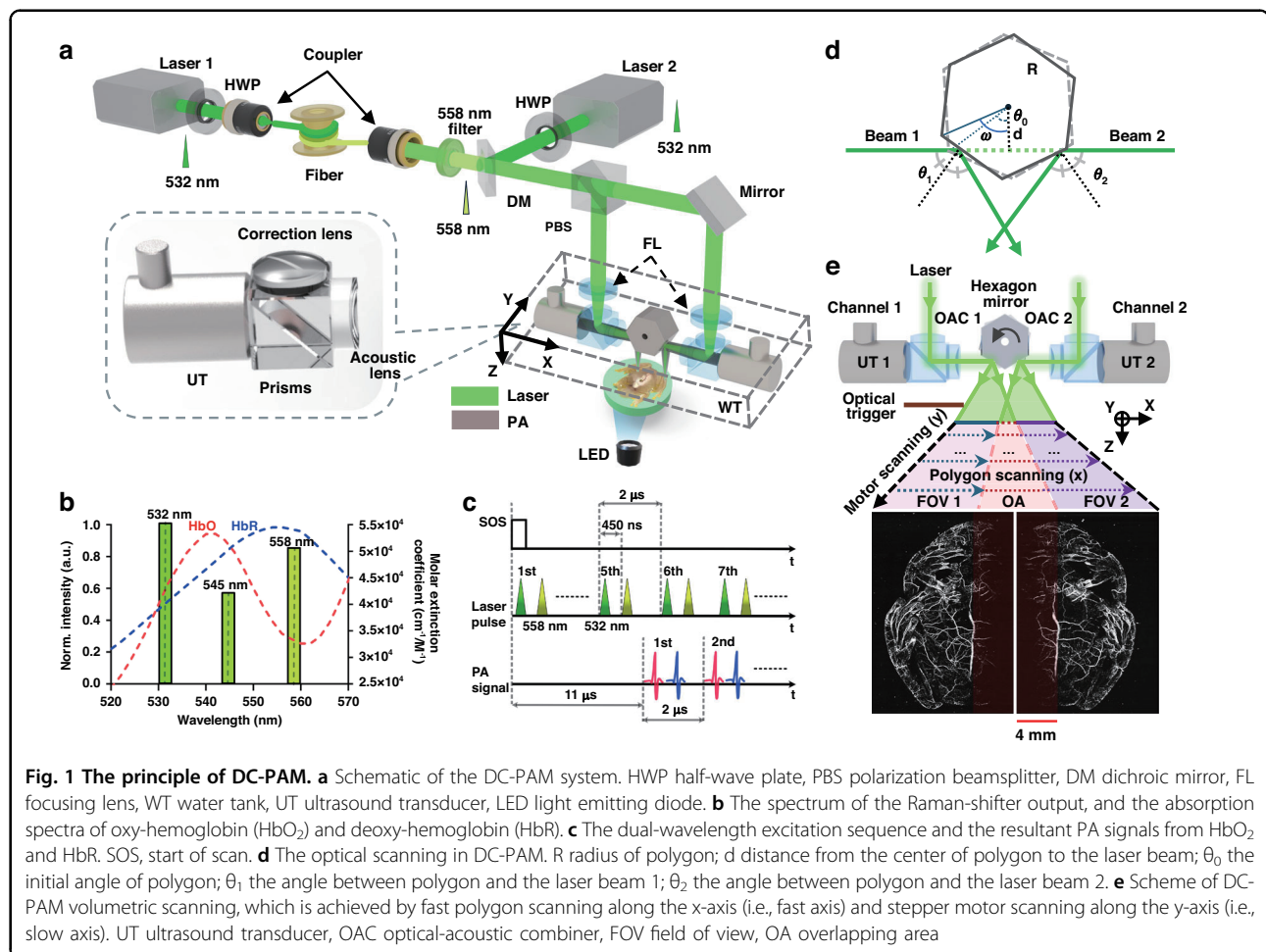
Results

Dual-channel PAM

The overview of the DC-PAM system is illustrated in Fig. 1a. Two pulsed lasers (SPFL-532-40, Spectra-Physics) are employed as the pump sources. A half-wave plate is placed after the laser output to adjust the polarization of the laser beams. One laser beam is coupled into a 25-m-long single-mode fiber (SMF-28, Thorlabs) to generate the 558-nm light via the stimulated Raman scattering (SRS) effect (Fig. 1b). A bandpass filter (560-nm central

wavelength, 10-nm bandwidth, model #87-887, Edmund Optics Inc.) is used to pass the 558-nm wavelength. The other 532-nm beam is merged with the 558-nm beam using a dichroic mirror (T550lpxr-UF1, Chroma Technology Corp). The 558-nm laser pulse lags the 532-nm pulse by 450 ns (Fig. 1c). The merged beams are split into two sub-beams by a polarization beamsplitter (CCM5-PBS201, Thorlabs).

Figure 1e shows the compact design of the DC-PAM imaging head. The two optical excitation sub-beams are delivered to two optical-acoustic combiners (OAC), which are placed on the opposite sides of the polygon scanner. The OAC includes a correction lens, a focusing lens, two right-angled prisms, a 30 MHz ultrasound transducer (V213-BC-RM, Olympus), and an acoustic lens. The detected PA signals are amplified (AML0040-00-6010, Wenteq Microwave) with a gain of 60 dB before being digitized at 250 MHz (ATS9350, AlazarTech). No signal averaging is performed. The detailed structure of the DC-PAM imaging head is shown in Fig. 1a and Fig. S1. To protect electrical components, including the polygon scanner, we sealed the imaging head in a 3D-printed



chamber with a shaft and ball bearings at both ends to ensure smooth scanning. Each polygon facet is $7.0 \times 10 \text{ mm}^2$, providing a 14-mm scanning range per channel. The overlapping area is $\sim 4 \text{ mm}$ near the edge of each scanning range, resulting in a total effective scanning range of 24 mm ³⁹.

Each laser pulse generates a one-dimensional time-resolved PA signal, and volumetric imaging is achieved through fast polygon scanning along the x -axis (fast axis) and stepper motor scanning along the y -axis (slow axis) (Fig. 1e). To synchronize the fast-axis with the slow-axis, a multimode fiber is mounted at the beginning of the line scanning to provide a “start of scan” signal. Since the time interval between two laser pulses is shorter than the PA signal travel time from the target to the ultrasound transducer, we precisely synchronize the laser firing and data acquisition with the scanning (Fig. S5). We use a 450-ns interval between the dual-wavelength laser pulses at 532 and 558 nm (Fig. 1c), which corresponds to a spatial separation of approximately 0.675 mm in soft tissue with a speed of sound of 1500 m/s . This spatial separation is larger than the optical depth of focus ($\sim 0.221 \text{ mm}$), avoiding overlapping between the two-wavelength PA signals within a single A-line for accurate spectral unmixing, even for a slightly uneven tissue surface (Fig. 1b).

The dual-beam steering by the polygon scanner (namely Channel 1 and Channel 2) is illustrated in Fig. 1d, Supplementary Video 1, and Fig. S1. The steering angles of the two channels follows:

$$\begin{cases} \theta_1 + \theta_2 = 60^\circ (60^\circ - \theta_0 < \omega < \theta_0) \\ \theta_1 + \theta_2 = 120^\circ (\omega \geq \theta_0) \end{cases}$$

Where R is the radius of the polygon; d is the orthogonal distance between the incident laser beam and the center of polygon; $\theta_0 = \cos^{-1}(\frac{d}{R})$ is the initial angle of the polygon; θ_1 and θ_2 are the incident angle of the laser beam relative to the polygon facet, respectively for Channel 1 and Channel 2; ω is the scanning angle of the polygon. There is practically no interference between the two channels. Although an overlapped scanning region may occur when the sum of θ_1 and θ_2 is 60° , the optical foci of the two channels are designed such that their respective depths of field do not overlap, and thus the resultant PA signals effectively do not interfere. Furthermore, each channel uses an independent ultrasound transducer, which, combined with the spatial separation of the optical foci, efficiently prevents PA signal interference between the channels (Fig. S1).

For phantom experiments, DC-PAM can achieve a maximum time-resolved A-line rate of 2 MHz at 532 nm and a B-scan frame rate of 500 Hz (Fig. S3d). For in vivo experiments, we usually use a laser repetition rate of

500 kHz , a B-scan frame rate of 300 Hz , and an average scanning step size of $8.4 \mu\text{m}$. This adjustment is to minimize the potential photothermal damage to the tissue over prolonged imaging sessions⁴¹. The lateral resolution and axial resolution are estimated to be 7.5 and $33 \mu\text{m}$, respectively (Fig. S2). With a step size of $5 \mu\text{m}$ for the slow axis and a B-scan rate of 300 Hz for the fast axis, the total scanning time for an FOV of $22.5 \text{ mm} \times 24 \text{ mm}$ is $\sim 15 \text{ s}$. The distortion of the images due to the rotatory scanning has been corrected by using a depth correction method⁴². For each channel, the optical excitation beam was co-axially aligned with the acoustic detection axis of the ultrasound transducer to maximize the signal-to-noise ratio (SNR). To minimize inter-channel misalignment during simultaneous scanning, the dual-channel probe housing was fabricated using high-precision CNC machining. Given that the axial resolution of the ultrasound transducer is $\sim 33 \mu\text{m}$, the machining precision introduces negligible alignment error. When inter-channel misalignment does occur due to mechanical drifting, image registration techniques can be applied in post-processing to correct for spatial offsets.

Figure 2 and Fig. S4 show the data processing workflow, including facet registration, deep-learning-based upsampling, and frame stabilization. The polygon scanner immersed in water suffers from the wobbling motion due to the water damping force, along with minor size variations among its six facets. This results in misaligned scanning trajectories and distorted images (Fig. 2a, b). Each facet can scan consistently (Fig. 2c), but the large step size and undersampling lower the image quality. To address these issues, we have developed a geometric-transformation registration method, which aligns scanning paths of different facets to correct facet misalignment. Applying this across all facets improves image quality (Fig. 2d, e).

Based on our previous work summarized in the Supplementary Information⁴⁰, we utilized a modified FD U-net model to upsample the registered DC-PAM images. The model was trained using the high-resolution PAM images of mouse brain vasculature⁴³, which were numerically downsampled to simulate undersampling. This deep-learning-based upsampling method corrects the undersampling without causing blurring or jagged edges like traditional interpolation methods, and avoids creating spurious fake structures (i.e., “hallucinations”)^{44–46}. It complements our facet alignment by removing minor misalignment artifacts and improves image quality without increasing the laser pulse repetition rate or risking the thermal damage (Fig. 2f). For further evaluation, Fig. 2g–j illustrate that the enhancement in vessel structures contributes to improved quantitative analysis. Figure 2k, l displays final DC-PAM images of an

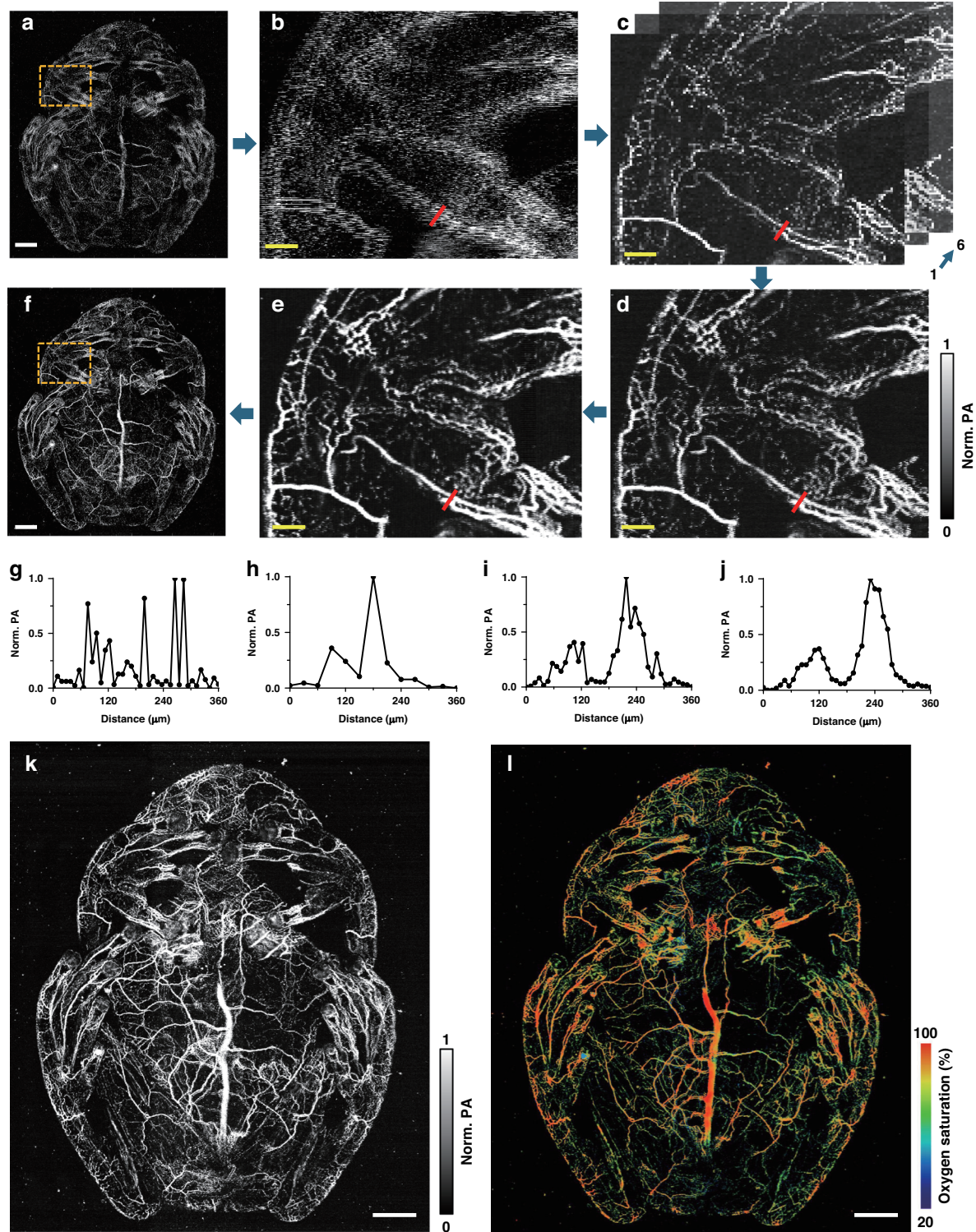
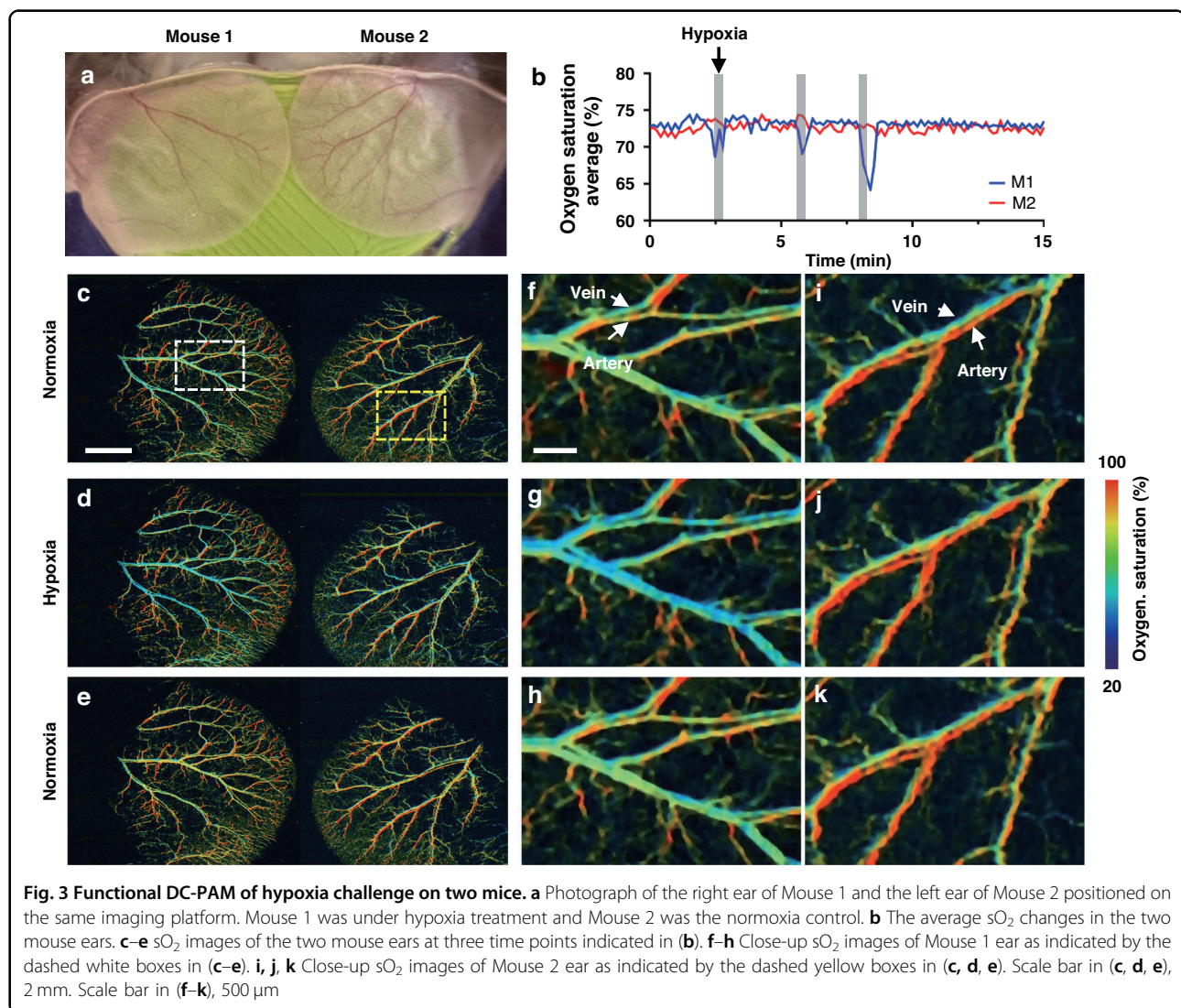


Fig. 2 Image registration and deep-learning-based upsampling. **a** The misaligned and undersampled PA image of the glassfrog vessels, assembled from all six facets of the polygon scanner. **b** Close-up image of the dashed box region in **(a)**, showing the misalignment of facets. **c** Close-up images generated by every facet. **d** Close-up assembled image after deep-learning-based upsampling. **e** Close-up assembled image after facet alignment. **f** The whole-body glassfrog image after facet alignment and deep-learning upsampling. **g–j** The intensity profiles of the representative vessels marked by the red lines in **(b–e)**. **k** The vasculature image of an awake glassfrog with facet alignment and upsampling. **l** The oxygen saturation image of the same glassfrog. Scale bar in **(a, f, k, l)**, 1 mm. Scale bar in **(b–e)**, 500 μm



awake glassfrog, showing the whole-body vasculature network and vessel-by-vessel sO_2 map.

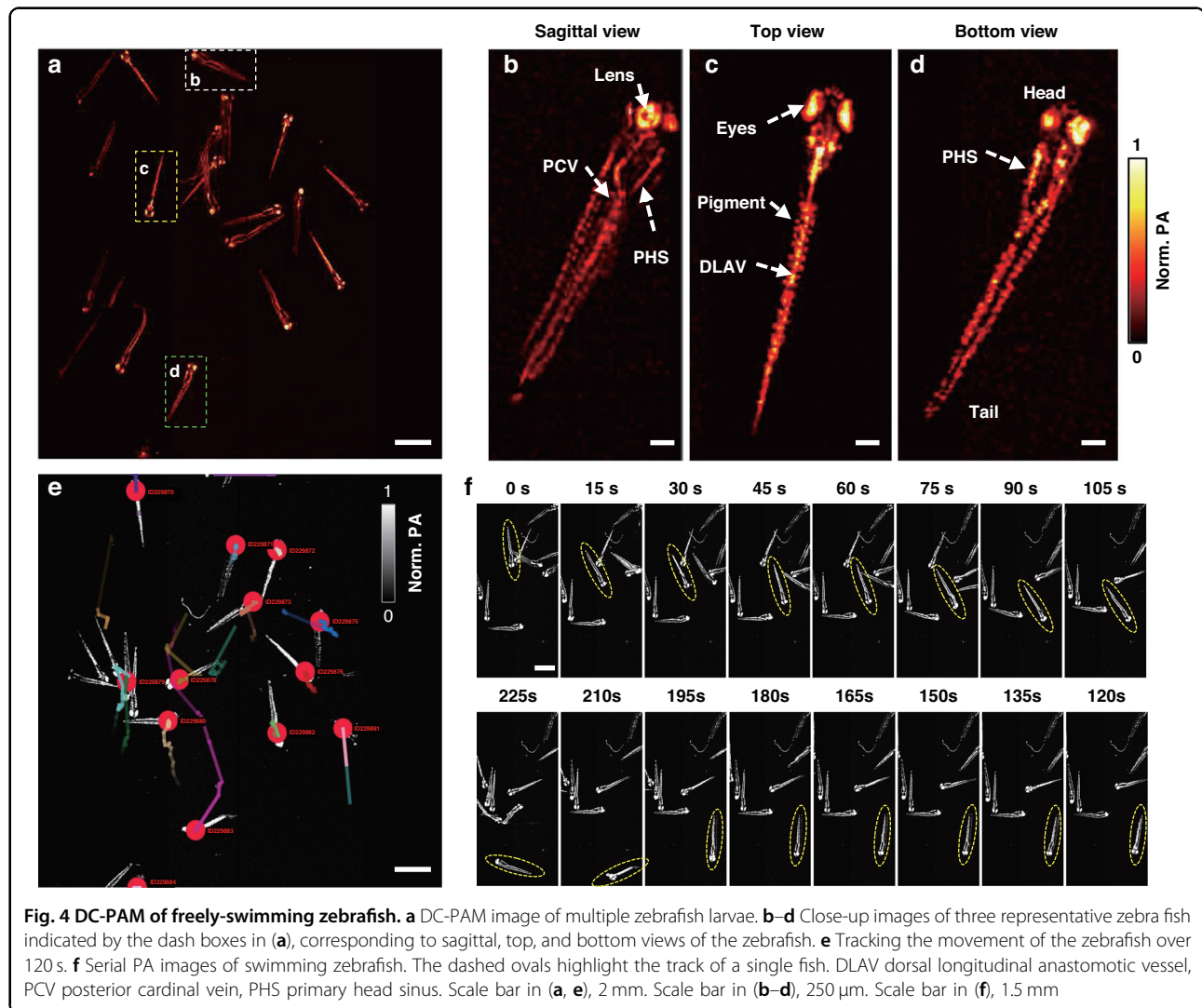
Simultaneous functional imaging of two mice

In preclinical research, studying two mice simultaneously, with one serving as a control and the other undergoing treatment, allows for direct comparative analysis of physiological responses under the same experimental conditions. This approach minimizes inter-subject variability caused by environmental fluctuations or instrument drift, thereby improving the reliability of functional imaging data. By leveraging DC-PAM's ultra-wide field of view, we imaged the ears of two mice simultaneously, with one mouse (namely Mouse 1, or M1) undergoing three cycles of hypoxia challenge and the other mouse (namely Mouse 2, or M2) serving as a control (Fig. 3a). Figure 3c–e present the sO_2 maps of the mouse ears under normoxia and hypoxia challenge. At

baseline (normoxia), there was a clear difference in sO_2 levels between the arteries (shown in red, indicating higher HbO_2 levels) and veins (shown in blue, indicating higher HbR levels). However, during the hypoxia challenge, the fraction of HbR increased, and the sO_2 levels became more uniform across the vessels. DC-PAM tracked the dynamics of three hypoxia cycles, as shown in Fig. 3b and Supplementary Video 2.

Freely-swimming zebrafish

Zebrafish is a popular animal model for toxicological studies, developmental biology, and drug screening, due to their genetic similarity to humans and their transparent bodies during early stages, which allow for direct imaging of internal structures. Studying freely-swimming zebrafish provides a powerful approach to investigating physiological dynamics in a natural, unrestrained condition. Here, we applied DC-PAM to record zebrafish larvae freely

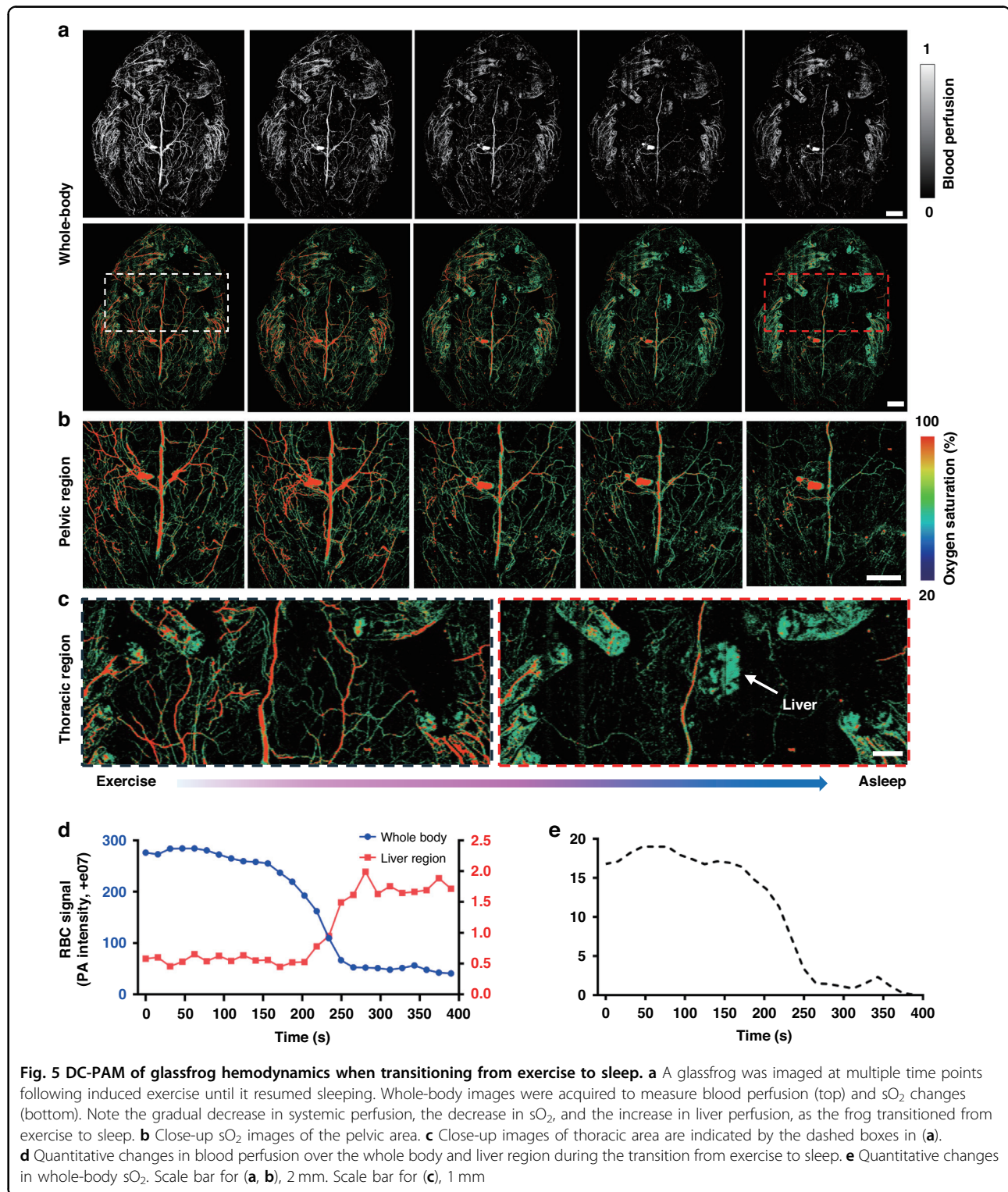


swimming in a 22.5 mm $\times$ 24 mm open arena. Figure 4a shows the images of anesthetized 8-day post-fertilization (dpf) fish. From the close-up images (Fig. 4b–d), we clearly observed anatomical structures of zebrafish, such as the retinal pigment epithelium of the eye, body pigments, anterior and posterior cardinal veins, primary head sinus, and the dorsal longitudinal anastomotic vein. We tracked 15 fish using Trackmate ImageJ, an open-source tracking algorithm (Supplementary Video 3), over a total period of 900 seconds. The high throughput of DC-PAM allowed us to observe fine details over a very wide FOV, capturing the dynamic swimming events, as shown in Fig. 4e and Supplementary Video 4. Unlike traditional PAM methods that require immobilization, DC-PAM provides the real-time observation of zebrafish development in response to environmental or physiological stimulation, and contributes to our understanding of their cardiovascular

function, metabolic regulation, and group behavior in relatively natural conditions.

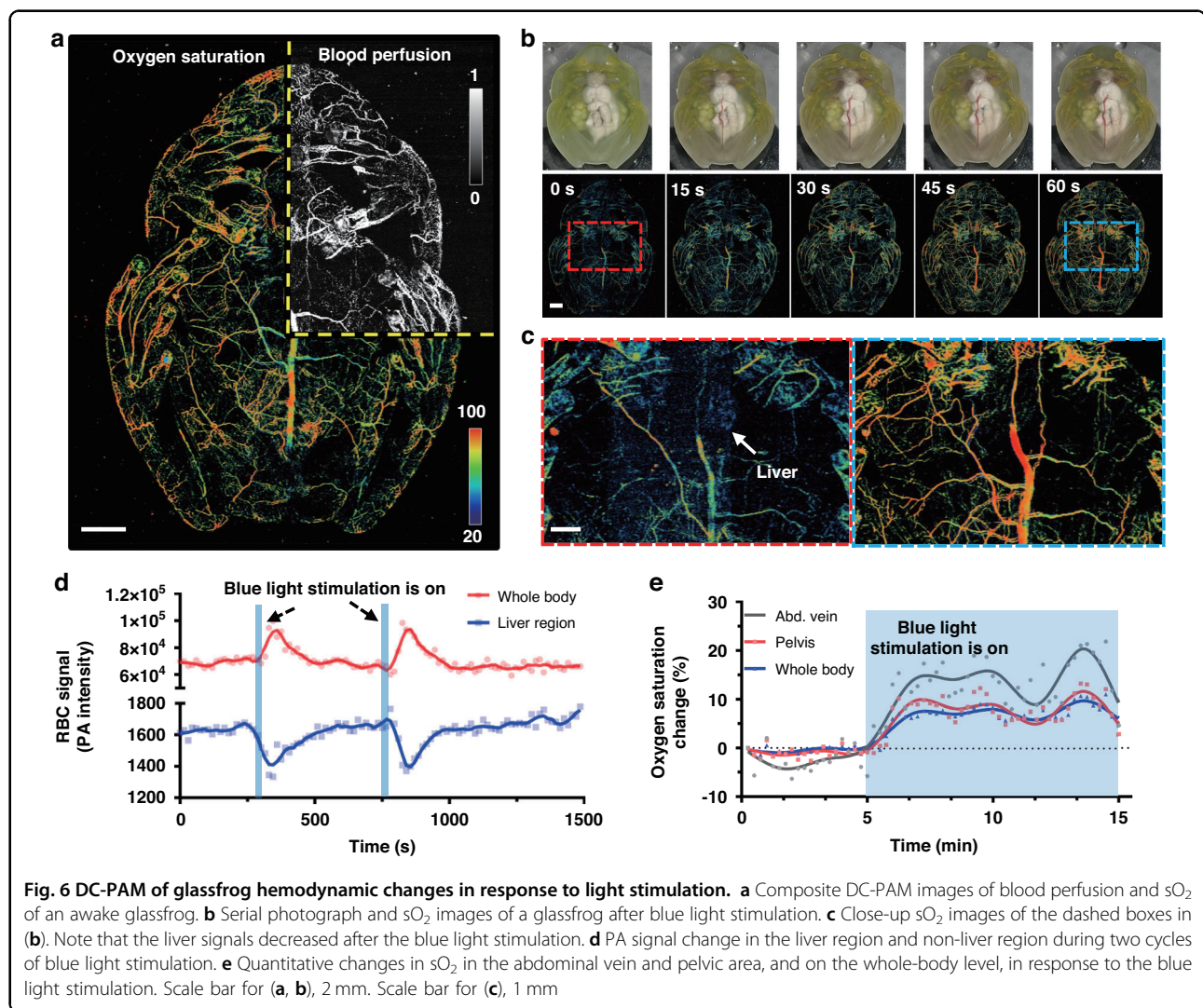
Glassfrog (*Hyalinobatrachium fleischmanni*)

Studying the hemodynamics of glassfrogs provides unique insights into their extraordinary ability to regulate blood perfusion and maintain transparency during sleep, a rare physiological adaptation with potential biomedical implications¹. Understanding these hemodynamic mechanisms not only sheds light on the evolutionary strategies of glassfrog transparency but also offers a comparative model for studying circulatory adaptations, blood pooling mechanisms, and potential applications such as thrombosis, vascular disorders, and micro-circulatory regulation. Here, we used DC-PAM to conduct functional whole-body imaging of glassfrogs to document their mechanism for removing most red blood



cells from the circulation by sequestering them in their liver during sleep (Fig. 5 and Supplementary Video 5). Figure 5 shows whole-body blood perfusion and sO₂ images at different time points as the glassfrog resumed sleep after exercise stimulation. Blood perfusion, along

with sO₂, gradually decreased, while the signal from their liver increased (as shown in Fig. 5d), confirming the findings that glassfrogs can hide their red blood cells in the liver to maintain transparency during sleep¹.



We further investigated the glassfrog's response to blue light stimulation, which is known to relax blood vessels in vertebrates⁴⁷. Figure 6a shows the DC-PAM image of an awake female glassfrog. Photographs and corresponding sO_2 images illustrate increased blood perfusion in the systemic circulation (Fig. 6b) and decreased liver signals (Fig. 6c) upon blue light stimulation. These results indicate that blue light can act as a stimulus that induces the release of red blood cells from the liver back to the systemic circulation, suggesting a similar mechanism to the transition from exercise to sleep (Fig. 5). To evaluate the reversibility of the hemodynamic changes induced by blue-light stimulation, we quantified blood perfusion in the liver region and the whole-body non-liver region during two 30-s stimulations, as shown in Fig. 6d. The results suggest a reversible mechanism of pooling and release of red blood cells in and out of the liver. To further investigate the changes in sO_2 induced by blue light, we imaged a glassfrog during a 10-min stimulation event

(Fig. 6e and Supplementary Video 6) and quantified the sO_2 values in the abdominal vein, pelvic area, and the entire body. In all cases, sO_2 increased by ~ 7 –20% over the baseline. These results highlight the dynamic and reversible physiological changes induced by blue light stimulation, which may resemble the frog's natural transition from between sleeping and awakening.

Discussion

In summary, our dual-channel photoacoustic microscopy (DC-PAM) system achieves a balanced combination of ultra-wide field of view ($22.5 \times 24 \text{ mm}^2$), high-speed functional imaging (B-scan rate $>500 \text{ Hz}$), and high spatial resolution ($\sim 7.5 \mu\text{m}$ lateral resolution and $\sim 33 \mu\text{m}$ axial resolution). DC-PAM is a useful functional imaging technology for large-scale imaging applications. DC-PAM leverages a water-immersible polygon scanner that concurrently steers two independent excitation laser beams and the resultant ultrasound waves. Consequently,

DC-PAM allows for high-throughput, high-fidelity imaging of whole biological systems, as demonstrated in zebrafish, mice, and glassfrogs. Particularly, while our previously reported polygon-based PAM was used to image only the abdominal region of the glassfrogs¹, DC-PAM was capable of imaging the whole-body glassfrogs at a high speed. This improvement allowed for a detailed study of the frog's hemodynamic changes as they transitioned from exercise to sleep. The results align well with our previous study, which demonstrated that glassfrogs concealed their blood in the liver during sleep while maintaining their transparency¹. Additionally, we have observed that blue light can reversibly increase glassfrog's systemic perfusion in a manner similar to the process of awakening, and this systemic perfusion coincides with a decrease in the red blood cells inside the liver, suggesting an interesting mechanism of pooling and releasing red blood cells. In these studies, the expanded FOV not only improves the imaging throughput but also enables new biological insights that would be challenging to study using conventional PAM systems. These results collectively demonstrate that DC-PAM provides a promising method to investigate functional, dynamic, and physiological changes on different small animal models.

However, the current DC-PAM system presents certain limitations. Firstly, our setup requires water immersion of the polygon scanner, which significantly constrains both the maximum imaging speed and image quality due to the damping force and resistance of water. In contrast, polygon scanners operating in air offer greater stability and faster scanning than their water-immersed counterparts. To address this issue, cylindrically focused ultrasound transducers with a large detection aperture (> 20 mm) coupled with rapid optical scanning can be employed to eliminate the need for water immersion. Second, the current system relies on a motorized scanning stage to translate the sample, which limits the volumetric imaging rate. To address this, a faster linear motorized stage could be implemented to improve the scanning speed. However, while this solution may increase the volumetric imaging rate, translating the sample at a high-speed presents challenges for in vivo experiments. Therefore, further optimization is needed to balance the scanning speed with the need for stable imaging for in vivo applications. Third, the current dual-channel configuration has a 4-mm overlapping area, $\sim 28\%$ of the single-channel scanning range. This overlapping is primarily resultant from the hexagon scanner's geometry and large scanning angle. It reduces the effective total scanning range, as some laser pulses are wasted to illuminate the overlapped scanning range. To mitigate this issue, an octagon scanner can be explored to provide dual-beam steering. Compared with the hexagon scanner, the octagon scanner, with its larger number of facets, facilitates smoother transitions between

facets and reduces the scanning angle, which can improve the frame rate and expand the effective field of view. The octagon mirror reduces the scanning angle to approximately 90 degrees, leading to reduced beam path overlapping between channels. Importantly, this design does not require shortening the transducer's focal length and thus would not sacrifice the image quality.

In conclusion, the improved imaging capabilities of DC-PAM open new avenues for studying dynamic physiological processes in vivo with a large FOV and a high speed, paving the way for future applications in neurovascular research, developmental biology, oxygen metabolism, cardiovascular disease, and pharmacological screening. Further advancements in scanning stability, real-time image processing, and integration with complementary imaging modalities will further expand DC-PAM's potential in preclinical research.

Materials and methods

Animals

All animal experiments were conducted in compliance with Institutional Animal Care and Use Committee (IACUC) guidelines at Duke University (Protocols: A009-20-01, A174-2108, and A069-22-04-25) and followed NIH guidelines for the care and use of laboratory animals.

Mice: The optical pulse energy at the ear surface was ~ 200 nJ per wavelength, with laser beams focused 250 μ m beneath the skin surface. Glassfrogs: The optical pulse energy used for glassfrog imaging was ~ 250 nJ per wavelength. Zebrafish: The optical pulse energy used for zebrafish imaging was ~ 50 nJ per wavelength.

Hexagon scanner

The six-facet hexagon scanner was fabricated from BK-7 glass with aluminum-coated optical facets for high reflectivity ($>90\%$ at 532 nm). It features a clear facet aperture of 7 mm $\times$ 10 mm along the fast and slow scanning axes, respectively, and a polygon diameter of 14 mm. The hexagon scanner is powered by a direct-current electric motor (M1N10FB11G, Minebea Mitsumi), enabling smooth operation at speeds up to 10,000 rpm, corresponding to a B-scan rate of 1 kHz.

SRS-based dual-wavelength laser system

The stimulated Raman scattering (SRS)-based dual-wavelength laser system was constructed using a 25-m single-mode fiber (SMF-28, Thorlabs) aligned on a fiber alignment stage (561D-XYZ, Newport) with a bare fiber chuck (FPH-S, Newport). The fiber output was secured with a universal bare fiber terminator (BFT1, Thorlabs) and collimated via an achromatic fiber port (PAF2A-A10A, Thorlabs). The output laser spectrum was analyzed using an optical spectrometer (CCS175, Thorlabs).



Systemic hypoxia in mice

BALB/c mice (3–5 months old, male, 20–30 g) were used for in vivo imaging of the hypoxia challenge. To induce systemic hypoxia in mice, a 3D-printed nose cone was used to deliver regulated oxygen levels while maintaining anesthesia with 1.5% v/v isoflurane. Body temperature was regulated at 37 °C using a heating pad. Hypoxia was induced by reducing the oxygen concentration from 21 to 3% using flowmeters. The mice were first imaged under normoxic for 2 min, followed by a 30-s hypoxia (or stay under normoxia for the control mouse) exposure, with this cycle repeated three times.

Glassfrog experiments

Glassfrogs (*Hyalinobatrachium fleischmanni*) were housed in bioactive vivariums that mimicked their natural habitat, maintained at 22–25 °C, ≥70% relative humidity, and a 12-h light/dark cycle with frequent misting. They were fed a varied diet of crickets and fruit flies every 2 to 4 days. Two experimental assays were conducted:

Exercise-induced hemodynamic changes: Frogs were gently encouraged to jump within a plastic bucket for 5–10 min by tapping their posteriors. They were then transferred to Petri dishes and immediately imaged to capture the hemodynamic recovery process as they transitioned to a resting state.

Blue light-induced hemodynamic changes: Blue light exposure (455 nm, M455L4, Thorlabs) was used to assess its effect on vasodilation and systemic blood perfusion. Glassfrogs, known to concentrate red blood cells (RBCs) in their hepatic sinusoids while sleeping, were imaged before and after blue light stimulation to track RBC redistribution and circulatory dynamics.

Zebrafish experiments

Wild-type zebrafish (Ekkwill strain) were imaged at 8 and 14 days post-fertilization (dpf). The fish were housed in a recirculating AHAB system (Aquatic Habitats, Apopka, FL) at 28 °C under a 14-h light/10-h dark cycle. Larvae were bred in Danieau's embryo medium and anesthetized using Tricaine Methanesulfonate (MS222, 0.01%) at pH 7.2 to slow down movement during imaging. This method effectively reduces motion without long-term harm, as previously described⁴⁸.

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Author details

¹Department of Biomedical Engineering, Duke University, Durham, NC 27708, USA. ²Biology Department, Duke University, Durham, NC 27708, USA. ³Department of Biological Sciences, Vanderbilt University, Nashville, TN 37232, USA. ⁴Nicholas School of the Environment, Duke University, Durham, NC 27713, USA

Author contributions

V.T.N. designed and developed the DC-PAM system. V.T.N. and C.T. performed the experiments, analyzed and interpreted the data, and prepared the figures. C.T. and J.D. maintained the glassfrogs. N.J. contributed to the zebrafish study. T.V. programmed the software for system synchronization. S.-W.C. constructed the Raman laser. J.L., L.M., and A.D. developed the data registration and upsampling algorithms. C.T. and J.Y. conceived the project. J.Y. supervised the whole project. All authors contributed to the manuscript writing and editing.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

J.Y., T.V., and L.M. have financial interests with Lumius Imaging, Inc., which did not support this work. The remaining authors declare no competing interests.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41377-025-02114-3>.

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References

- Taboada, C. et al. Glassfrogs conceal blood in their liver to maintain transparency. *Science* **378**, 1315–1320 (2022).
- Feinberg, D. A. et al. Next-generation MRI scanner designed for ultra-high-resolution human brain imaging at 7 Tesla. *Nat. Methods* **20**, 2048–2057 (2023).
- Coupland, C. E. et al. High-resolution electron cryomicroscopy of V-ATPase in native synaptic vesicles. *Science* **385**, 168–174 (2024).
- Fang, Z. G. et al. Oxyhaemoglobin saturation NIR-IIb imaging for assessing cancer metabolism and predicting the response to immunotherapy. *Nat. Nanotechnol.* **19**, 124–130 (2024).
- Kim, J. et al. Super-resolution localization photoacoustic microscopy using intrinsic red blood cells as contrast absorbers. *Light Sci. Appl.* **8**, 103 (2019).
- Wang, L. V. & Hu, S. Photoacoustic tomography: in vivo imaging from organelles to organs. *Science* **335**, 1458–1462 (2012).
- Wang, L. V. & Yao, J. J. A practical guide to photoacoustic tomography in the life sciences. *Nat. Methods* **13**, 627–638 (2016).
- Yao, J. J. et al. High-speed label-free functional photoacoustic microscopy of mouse brain in action. *Nat. Methods* **12**, 407–410 (2015).
- Zhang, H. F. et al. Functional photoacoustic microscopy for high-resolution and noninvasive in vivo imaging. *Nat. Biotechnol.* **24**, 848–851 (2006).
- Kasatkina, L. A. et al. Deep-tissue high-sensitivity multimodal imaging and optogenetic manipulation enabled by biliverdin reductase knockout. *Nat. Commun.* **16**, 6469 (2025).
- Laufer, J. et al. In vitro measurements of absolute blood oxygen saturation using pulsed near-infrared photoacoustic spectroscopy: accuracy and resolution. *Phys. Med. Biol.* **50**, 4409–4428 (2005).
- Li, M. C., Tang, Y. Q. & Yao, J. J. Photoacoustic tomography of blood oxygenation: a mini review. *Photoacoustics* **10**, 65–73 (2018).
- Liu, C., Liang, Y. Z. & Wang, L. D. Single-shot photoacoustic microscopy of hemoglobin concentration, oxygen saturation, and blood flow in sub-microseconds. *Photoacoustics* **17**, 100156 (2020).
- Ning, B. et al. Simultaneous photoacoustic microscopy of microvascular anatomy, oxygen saturation, and blood flow. *Opt. Lett.* **40**, 910–913 (2015).
- Yao, J. J. et al. Label-free oxygen-metabolic photoacoustic microscopy in vivo. *J. Biomed. Opt.* **16**, 076003 (2011).



16. Sun, N. D. et al. Photoacoustic microscopy of vascular adaptation and tissue oxygen metabolism during cutaneous wound healing. *Biomed. Opt. Express* **13**, 2695–2706 (2022).
17. Cao, R. et al. Functional and oxygen-metabolic photoacoustic microscopy of the awake mouse brain. *Neuroimage* **150**, 77–87 (2017).
18. Cho, S. W. et al. Sounding out the dynamics: a concise review of high-speed photoacoustic microscopy. *J. Biomed. Opt.* **29**, S11521 (2024).
19. Maslov, K. et al. Optical-resolution photoacoustic microscopy for in vivo imaging of single capillaries. *Opt. Lett.* **33**, 929–931 (2008).
20. Zhang, H. F. et al. Imaging of hemoglobin oxygen saturation variations in single vessels in vivo using photoacoustic microscopy. *Appl. Phys. Lett.* **90**, 053901 (2007).
21. Rebling, J. et al. Dual-wavelength hybrid optoacoustic-ultrasound biomicroscopy for functional imaging of large-scale cerebral vascular networks. *J. Biophotonics* **11**, e201800057 (2018).
22. Nguyen, V. T. et al. Ultra-widefield photoacoustic microscopy with a dual-channel slider-crank laser-scanning apparatus for in vivo biomedical study. *Photoacoustics* **23**, 100274 (2021).
23. Pham, V. H. et al. Development of fast photoacoustic and ultrasound imaging system based on slider-crank scanner for small animals and humans study. *Expert Syst. Appl.* **206**, 117939 (2022).
24. Xie, Z. X. et al. Laser-scanning optical-resolution photoacoustic microscopy. *Opt. Lett.* **34**, 1771–1773 (2009).
25. Yuan, Y., Yang, S. H. & Xing, D. Optical-resolution photoacoustic microscopy based on two-dimensional scanning galvanometer. *Appl. Phys. Lett.* **100**, 023702 (2012).
26. Kim, J. Y. et al. High-speed and high-SNR photoacoustic microscopy based on a galvanometer mirror in non-conducting liquid. *Sci. Rep.* **6**, 34803 (2016).
27. Liao, T. Y. et al. Centimeter-scale wide-field-of-view laser-scanning photoacoustic microscopy for subcutaneous microvasculature in vivo. *Biomed. Opt. Express* **12**, 2996–3007 (2021).
28. Yao, J. J. et al. Wide-field fast-scanning photoacoustic microscopy based on a water-immersible MEMS scanning mirror. *J. Biomed. Opt.* **17**, 080505 (2012).
29. Kim, J. Y. et al. Fast optical-resolution photoacoustic microscopy using a 2-axis water-proofing MEMS scanner. *Sci. Rep.* **5**, 7932 (2015).
30. Chen, Q. et al. Ultracompact high-resolution photoacoustic microscopy. *Opt. Lett.* **43**, 1615–1618 (2018).
31. Lin, L. et al. Handheld optical-resolution photoacoustic microscopy. *J. Biomed. Opt.* **22**, 41002 (2017).
32. Jin, T. et al. Portable optical resolution photoacoustic microscopy (pORPAM) for human oral imaging. *Opt. Lett.* **42**, 4434–4437 (2017).
33. Jin, T. et al. Portable optical-resolution photoacoustic microscopy for volumetric imaging of multiscale organisms. *J. Biophotonics* **11**, e201700250 (2018).
34. Duma, V. F. & Podoleanu, A. G. Polygon mirror scanners in biomedical imaging: a review. In *Proc. SPIE 8621, Optical Components and Materials X* 175–183 (SPIE, 2013).
35. Lin, Y. C. et al. A compact and portable laser radioactive decontamination system using passive Q-switched fiber laser and polygon scanner. *Appl. Radiat. Isotopes* **153**, 108835 (2019).
36. Li, Y. X. et al. Flexible polygon-mirror based laser scanning microscope platform for multiphoton in-vivo imaging. *J. Biophotonics* **10**, 1526–1537 (2017).
37. Ji, Y. Y., So, B. H. & Kim, D. Y. High-speed time-domain characterization method for polygon scanners. *Measurement* **135**, 278–286 (2019).
38. Chen, J. B. et al. Wide-field polygon-scanning photoacoustic microscopy of oxygen saturation at 1-MHz A-line rate. *Photoacoustics* **20**, 100195 (2020).
39. Lan, B. X. et al. High-speed widefield photoacoustic microscopy of small-animal hemodynamics. *Biomed. Opt. Express* **9**, 4689–4701 (2018).
40. Zhu, X. Y. et al. Real-time whole-brain imaging of hemodynamics and oxygenation at micro-vessel resolution with ultrafast wide-field photoacoustic microscopy. *Light Sci. Appl.* **11**, 138 (2022).
41. Wang, L. D., Zhang, C. & Wang, L. V. Grueneisen relaxation photoacoustic microscopy. *Phys. Rev. Lett.* **113**, 174301 (2014).
42. Xu, Z. Q. et al. Visualizing tumor angiogenesis and boundary with polygon-scanning multiscale photoacoustic microscopy. *Photoacoustics* **26**, 100342 (2022).
43. DiSpirito III, A. Duke PAM dataset. *Zenodo* 105281/zenodo.4042170 (2020).
44. DiSpirito III, A. et al. Sounding out the hidden data: a concise review of deep learning in photoacoustic imaging. *Exp. Biol. Med.* **246**, 1355–1367 (2021).
45. Chandrasegaran, K., Tran, N. T. & Cheung, N. M. A closer look at fourier spectrum discrepancies for CNN-generated images detection. In *Proc. 2021 IEEE/CVF Conference on Computer Vision and Pattern Recognition* (IEEE, 2021).
46. Fuoli, D., Van Gool, L. & Timofte, R. Fourier space losses for efficient perceptual image super-resolution. In *Proc. 2021 IEEE/CVF International Conference on Computer Vision* (IEEE, 2021).
47. Sikka, G. et al. Melanopsin mediates light-dependent relaxation in blood vessels. *Proc. Natl Acad. Sci. USA* **111**, 17977–17982 (2014).
48. Raftery, T. D., Jayasundara, N. & Di Giulio, R. T. A bioenergetics assay for studying the effects of environmental stressors on mitochondrial function in vivo in zebrafish larvae. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **192**, 23–32 (2017).

