Imaging cerebral blood flow and oxygenation *in vivo*

Technical Journal Club

28. April 2015

Motivation

Study blood supply and oxygenation of the brain in vivo

Background: methods to functionally study blood oxygenation and vasculature

- Electrodes
- electron paramagnetic resonance methods e.g. fMRI
- phosphorescence lifetime–based two-photon microscopy (TPM)
- hemoglobin optical absorption–based methods
  - wide-field optical microscopy
  - Photoacoustic tomography (PAT)

Paper 1

NATURE METHODS | ARTICLE

Two-photon high-resolution measurement of partial pressure of oxygen in cerebral vasculature and tissue

Sava Sakadžić, Emmanuel Roussakis, Mohammad A Yaseen, Emiri T Mandeville, Vivek J Srinivasan, Ken Arai, Svetlana Ruvinskaya, Anna Devor, Eng H Lo, Sergei A Vinogradov & David A Boas

Affiliations | **Contributions** | **Corresponding authors**

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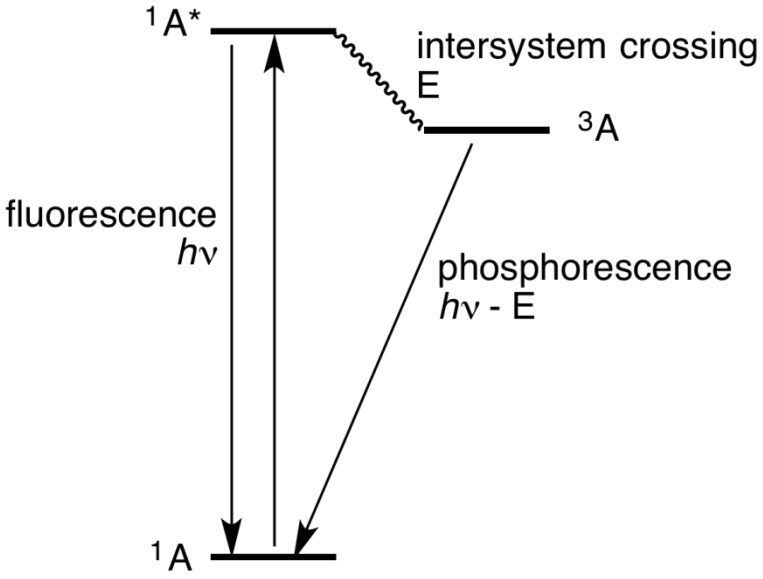
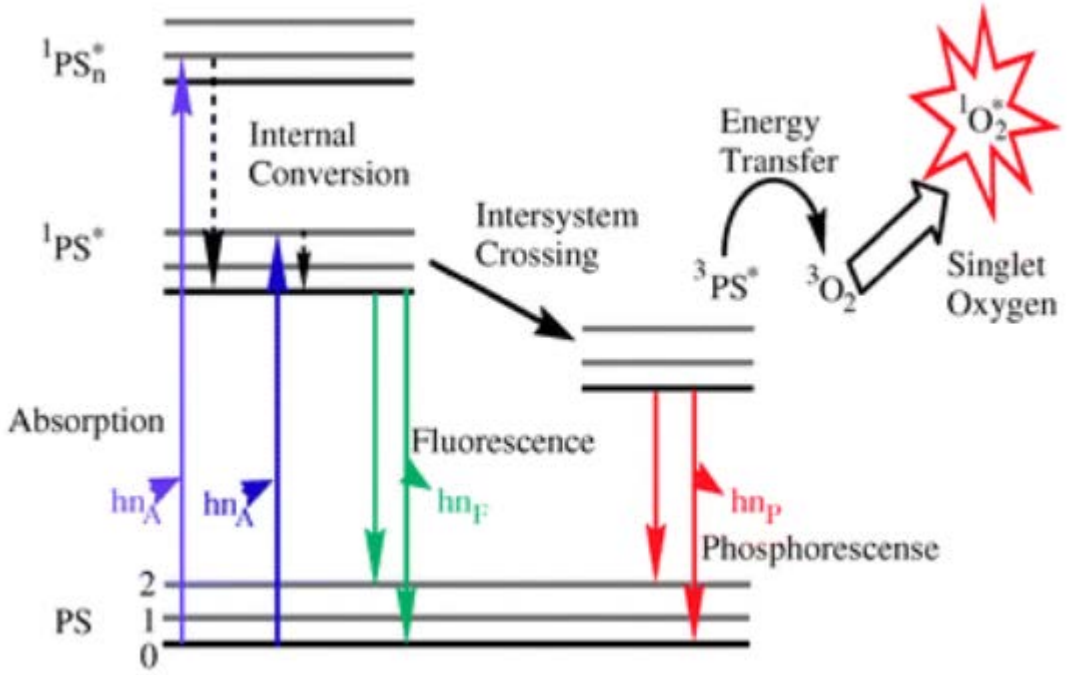
phosphorescence

- specific type of photoluminescence related to fluorescence but much slower

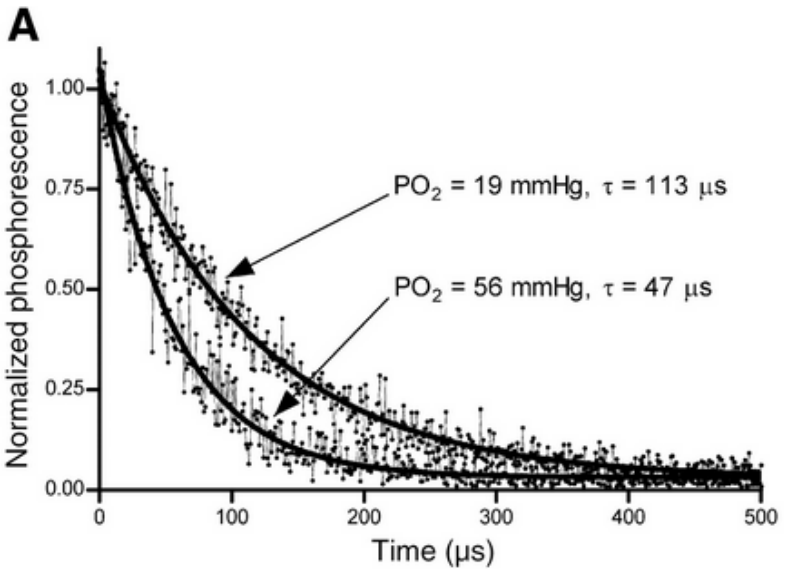


http://en.wikipedia.org/wiki/Phosphorescence

oxygen-dependent quenching of phosphorescence



$$\tau_0/\tau = 1 + kq \cdot \tau_0 \cdot pO_2,$$



https://photochemistry.wordpress.com/tag/excited-state/
http://jap.physiology.org/content/97/5/1962

Idea

- Combine oxygen-dependent quenching of phosphorescence with two-photon microscopy
  - Direct measurement of O2 partial pressure
  - Independent of optical properties of tissue
  - Precisely localized
  - Measurements not only on surface but also in the tissue possible

The problem

„Unfortunately, direct coupling of phosphorescence with two-photon microscopy is hampered by extremely low two-photon absorption crosssections of phosphorescent probes, necessitating very high excitation powers, long acquisition periods and/or exceedingly high probe concentrations“

The solution

- Specially designed two-photon–enhanced phosphorescent nanoprobe platinum porphyrin–coumarin-343 (PtP-C343)

Combined with

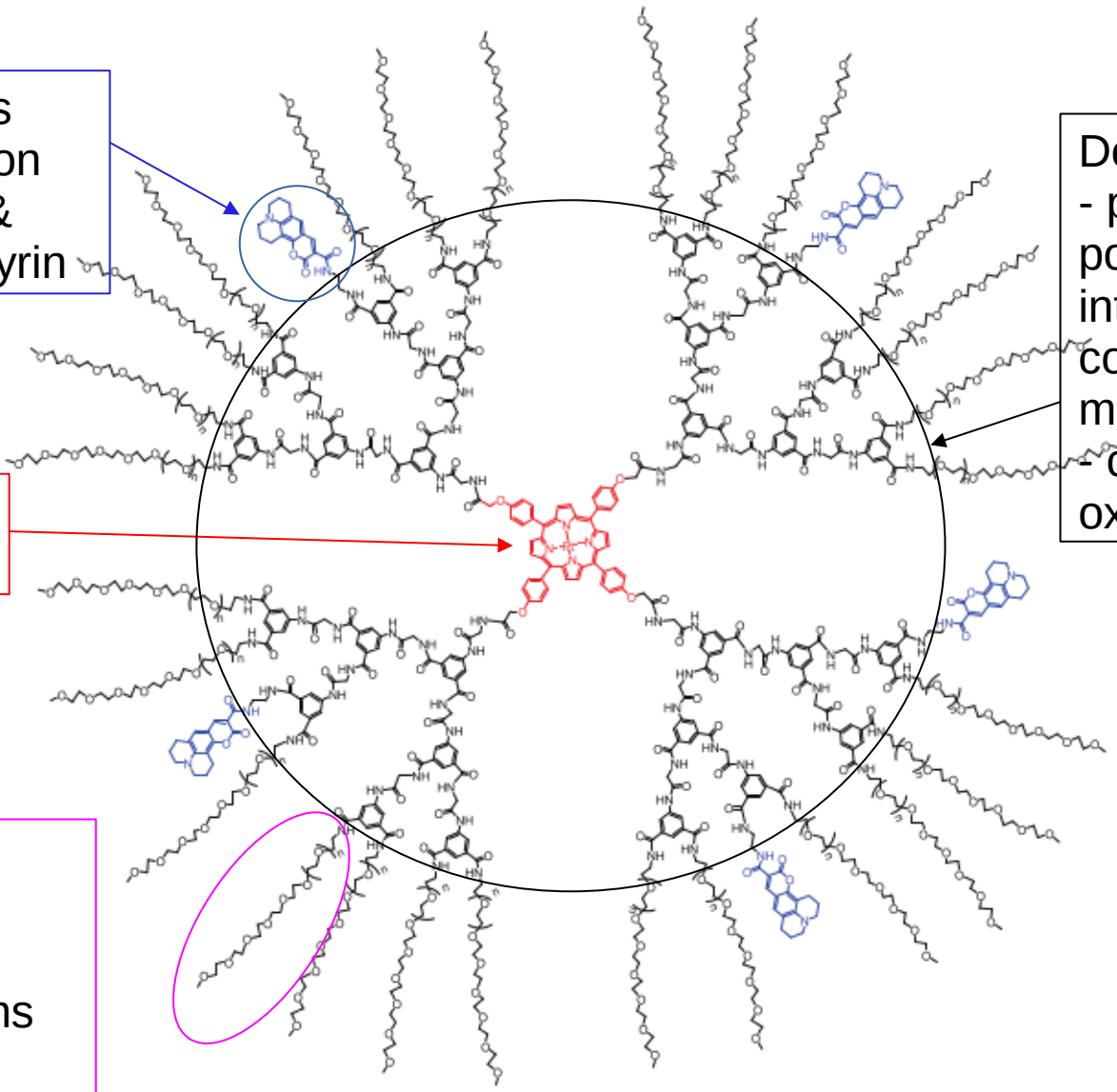
- optimized microscopy setup

PtP-C343

coumarin-343 units
- capture two-photon excitation energy & transfer it to porphyrin

Porphyrin (phosphorescent)

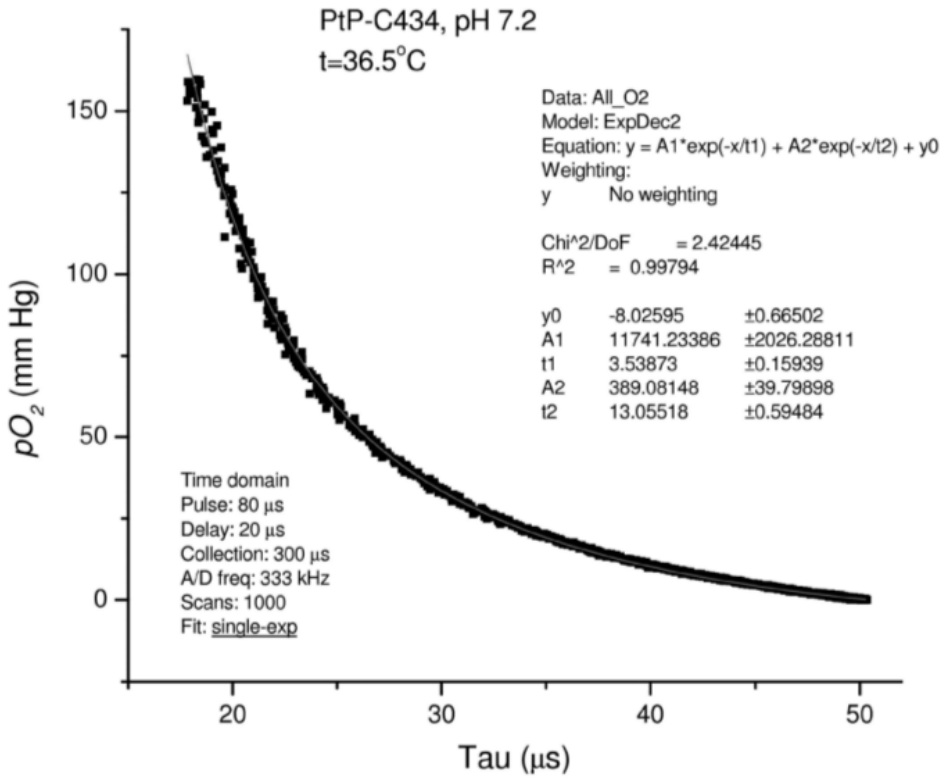
PEGylation
- ensures that the probe's signal is insensitive to proteins and other macromolecular solutes

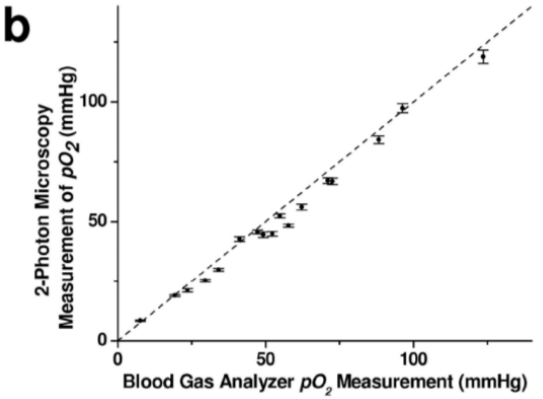


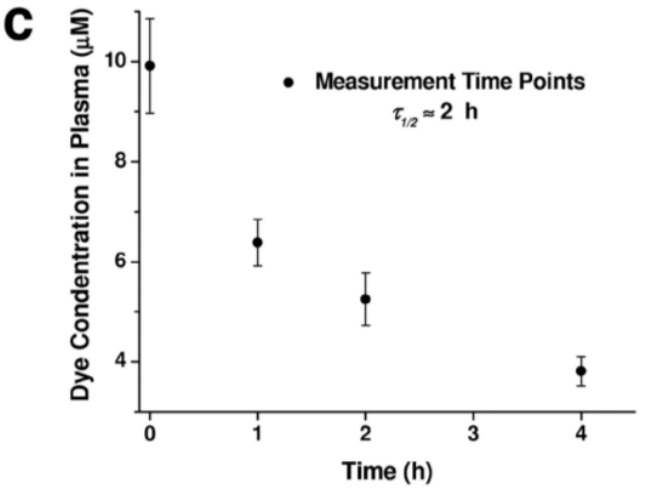
Dendrimer
- protects the porphyrin from interaction with components of the measurement system
- controls the rate of oxygen quenching

- Upon excitation, Pt porphyrin undergoes
- fast intersystem crossing into its triplet state and emits phosphor
- escence, which is quenched by molecular oxygen in a diffusion-
- controlled manner. Phosphorescence decay lifetime (typically
- several tens of microseconds) is inversely proportional to pO 2
- (via Stern-Volmer relationship), thus forming the signal for

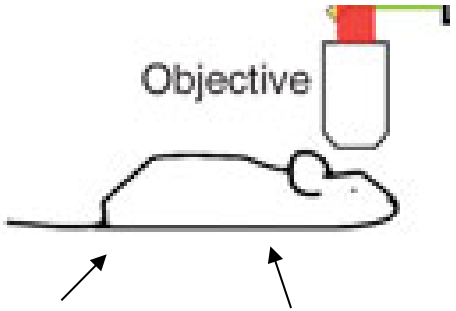
Calibration







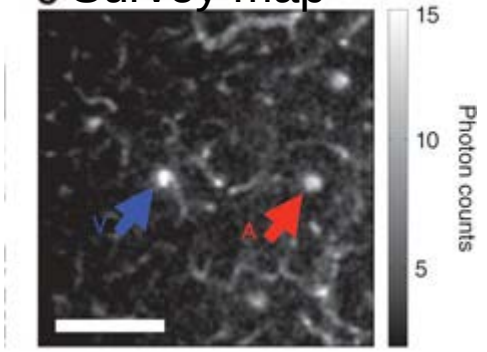
Experimental Setup



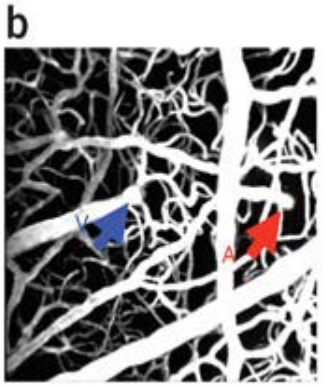
FITC-intravenously

PtP-C343
- intra-arterially
- intra-parenchymal

Raster-Scan



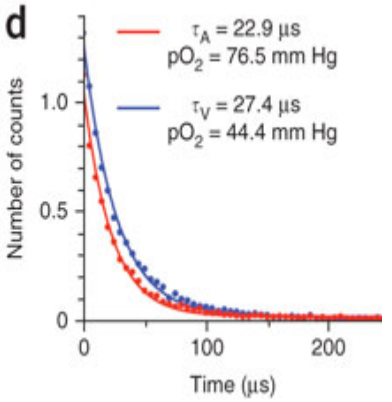
250x250px, 166 µm below the brain surface, 25s



500-2000x

20-80us excitation	300us detection

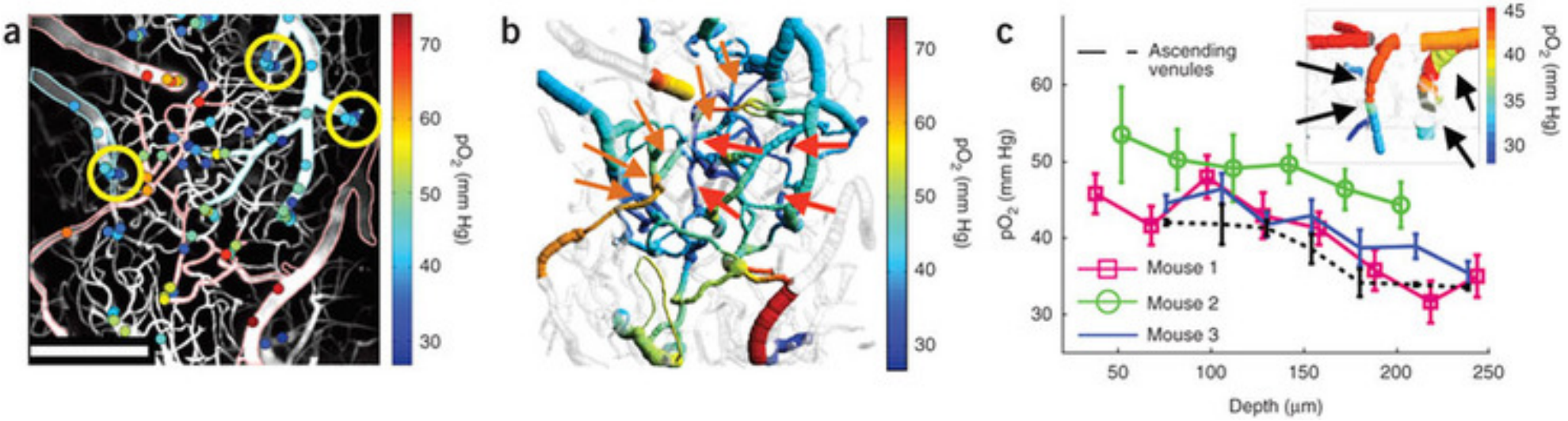
Emitting volume:
~2 µm lateral (x-y) and ~5 µm axial (z)



- 0.16–0.76 s per single-point pO 2
- entire vasculature stack: 30 min

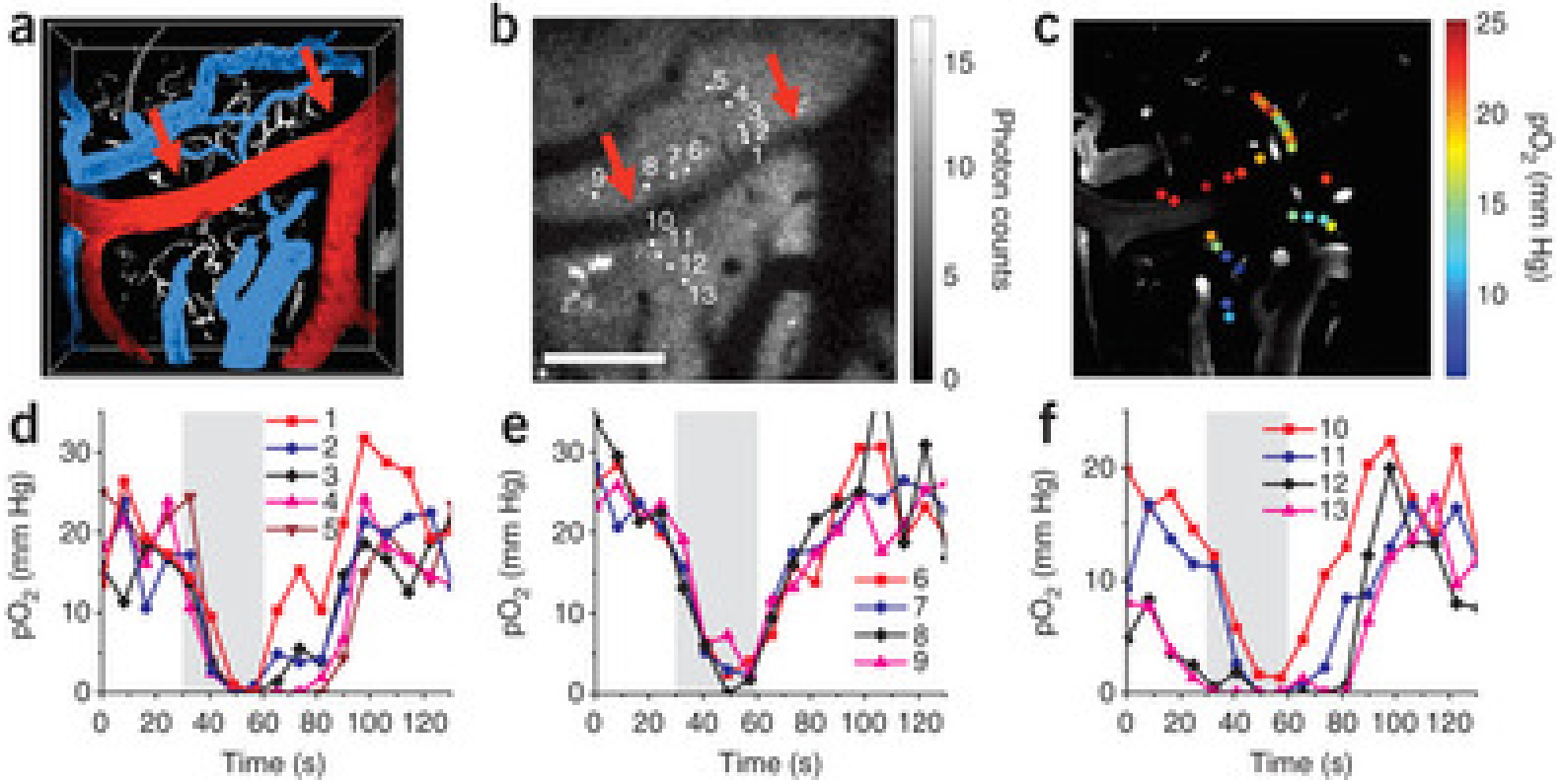
Oxygen tension in cortical microvasculature

approximately 100 pO_2 values in 30-μm steps down to 240 μm below the cortical surface in the mouse brain

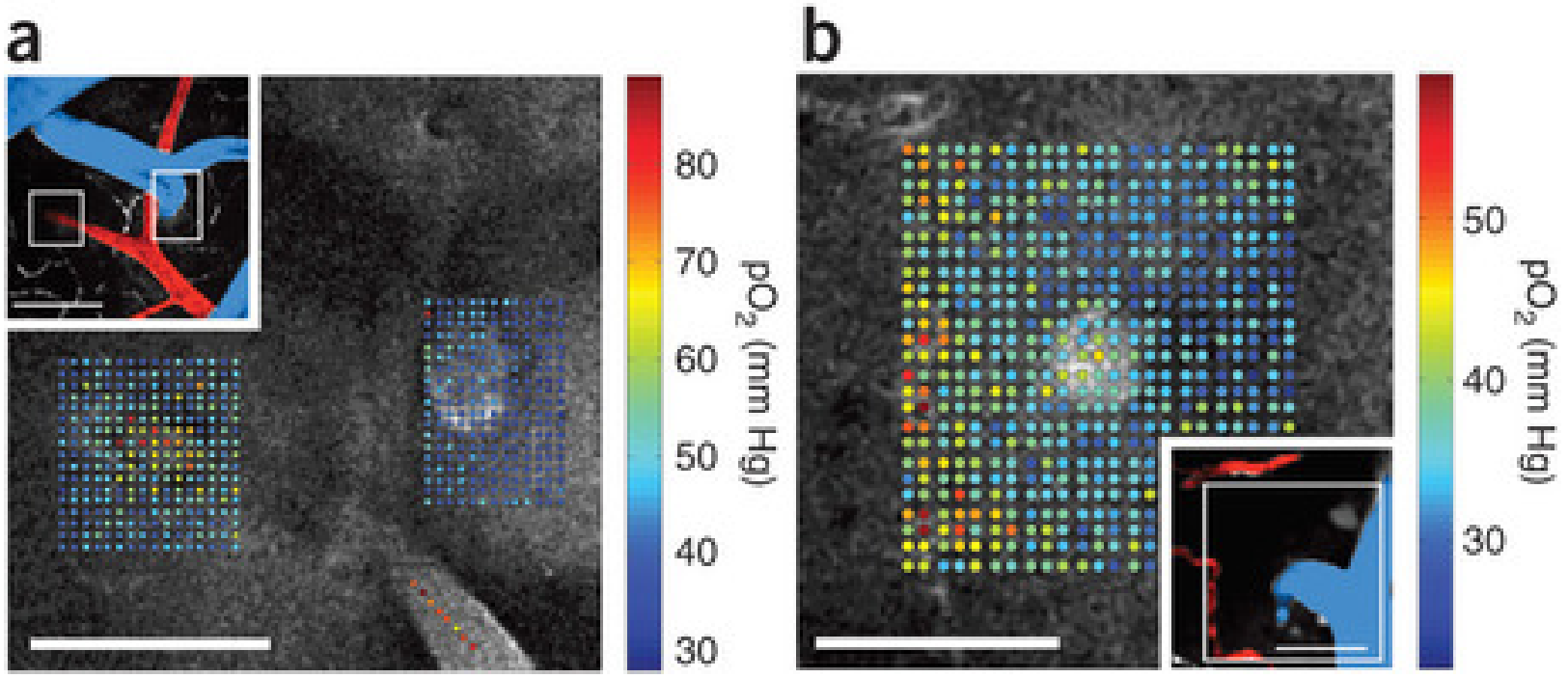


Oxygen tension in cortical tissue

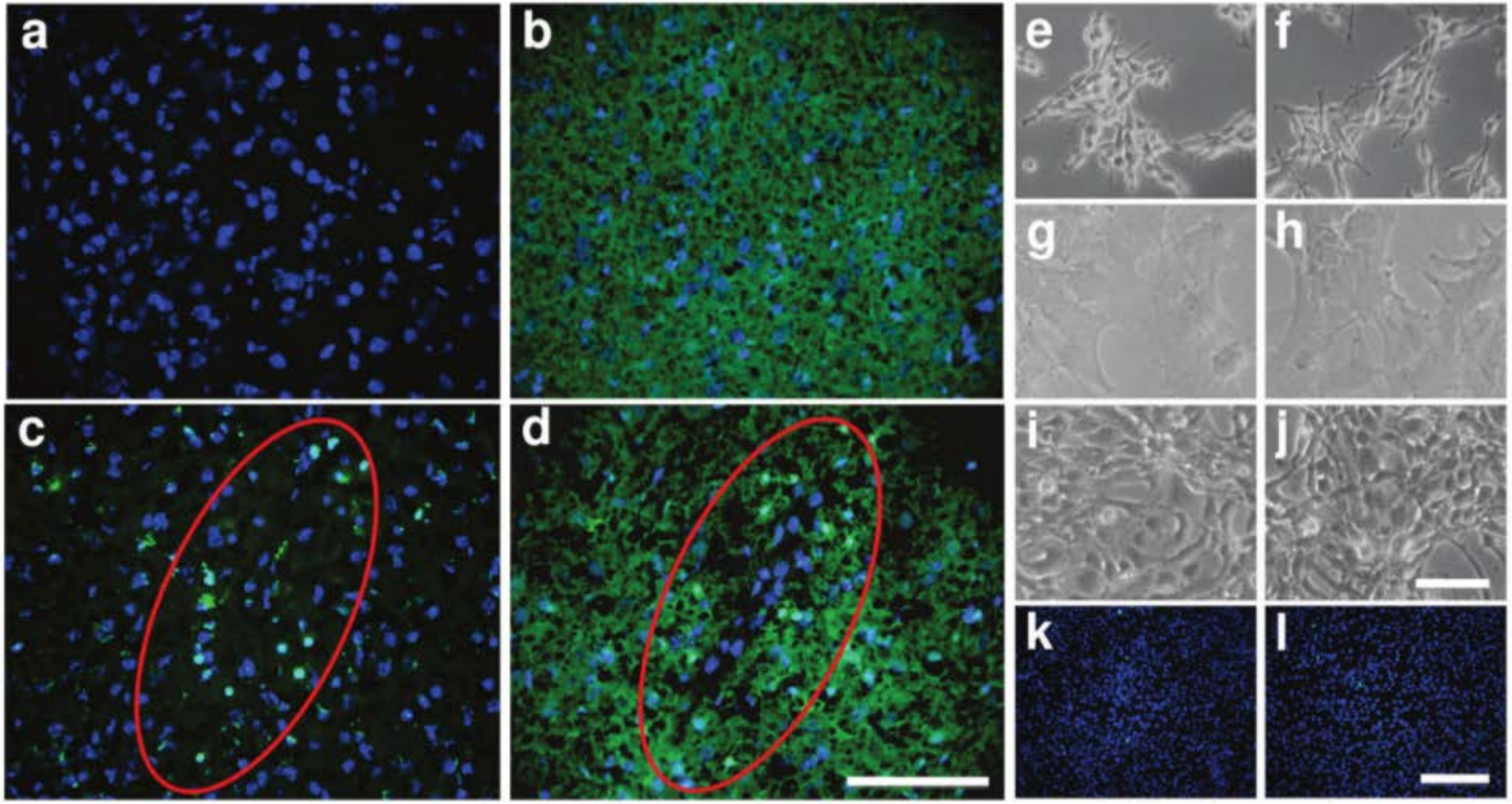
PtP-C343 injected directly into the interstitial space



Oxygen tension in cortical tissue and microvasculature



Assessment of phototoxicity



Summary

- Measurements of vascular and parenchymal oxygen pressure possible
- Low temporal resolution

Paper 2

NATURE METHODS | BRIEF COMMUNICATION

High-speed label-free functional photoacoustic microscopy of mouse brain in action

Junjie Yao, Lidai Wang, Joon-Mo Yang, Konstantin I Maslov, Terence T W Wong, Lei Li, Chih-Hsien Huang, Jun Zou & Lihong V Wang

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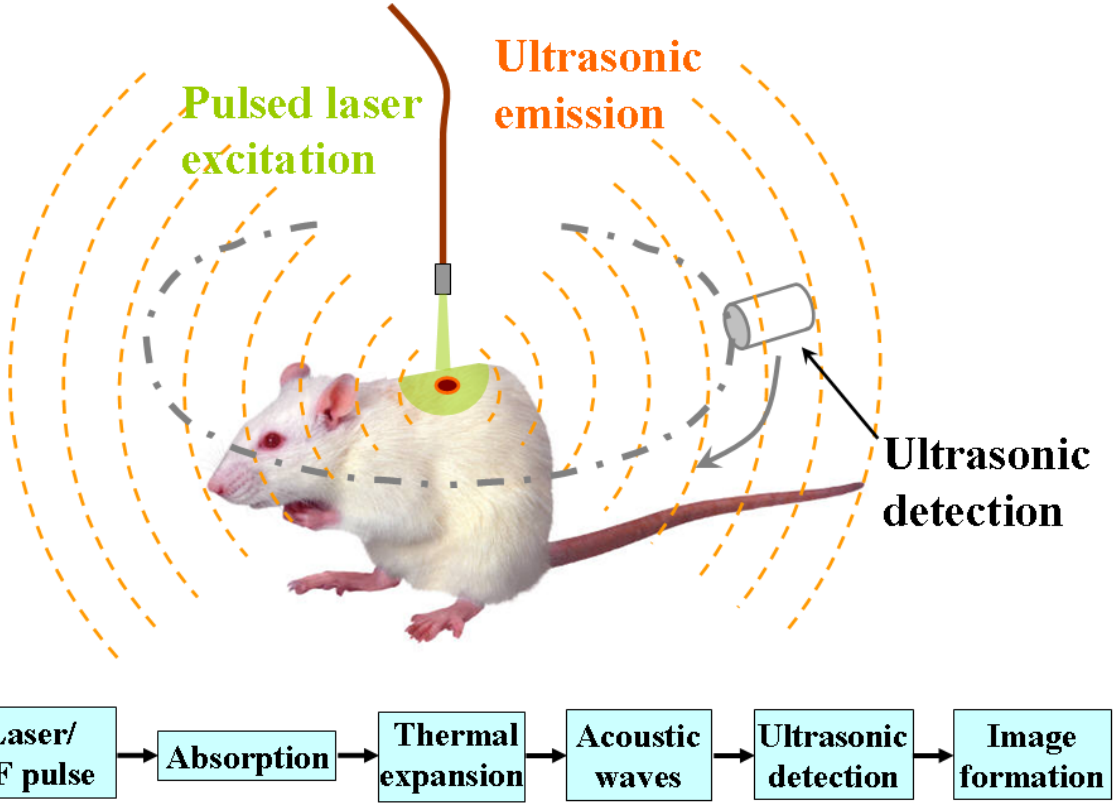
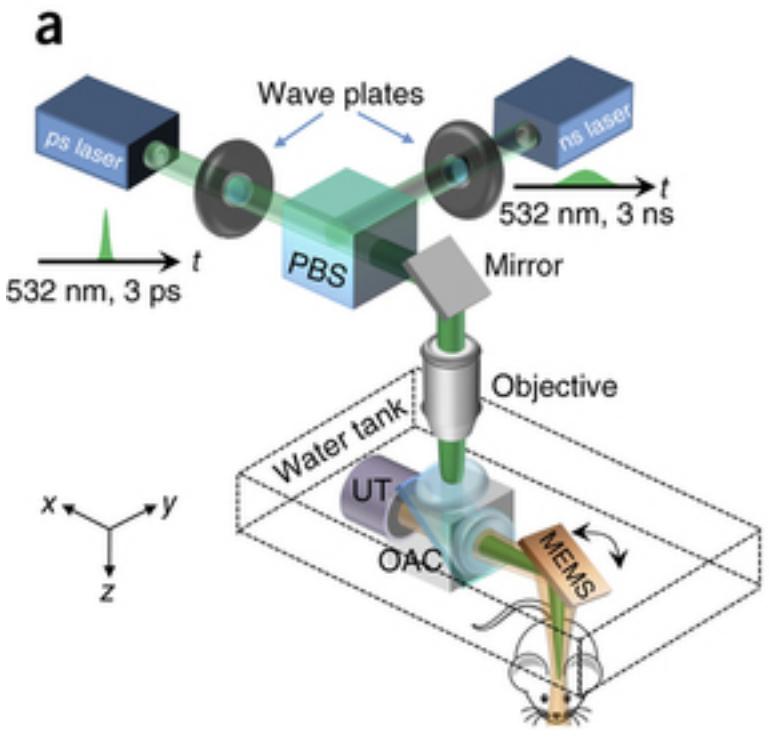
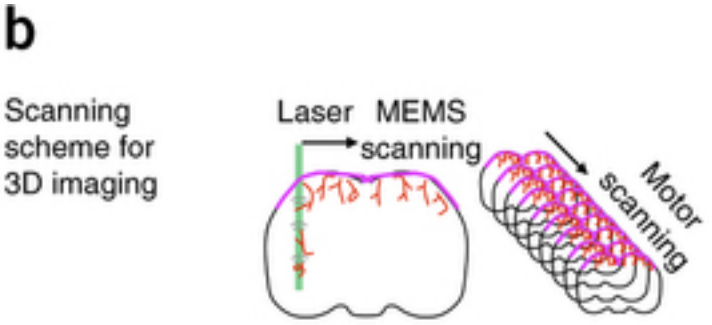
Background: photoacustic tomography



- PAT: detector is not focused. Mathematical reconstruction needed
- PAM: laser and detector focused, no reconstruction needed

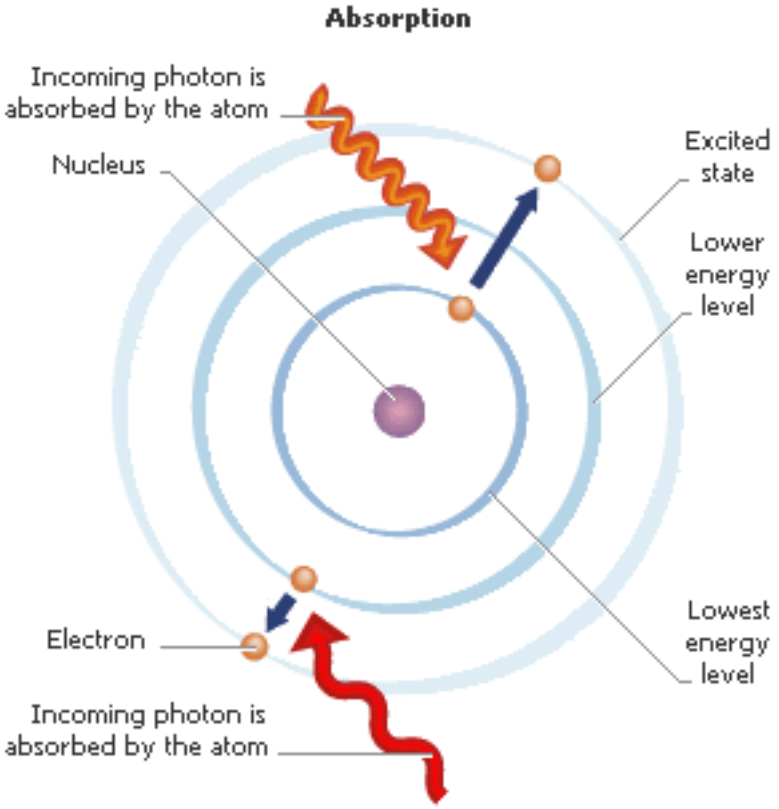
Photoacoustic microscopy (PAM)





- excitation laser beams and the detection acoustic axis confocally steered by a customized water-immersible MEMS (microelectromechanical system) scanning mirror
- lateral resolution (perpendicular to x) is ~3 μm
- Axial resolution is ~15 μm
- Temporal resolution:
  - Laser pulse repetition rate: 500kHz
  - 1D: 100kHz
  - 2D: 400Hz (3mm)
  - 3D: 1Hz (3x2mm^2)

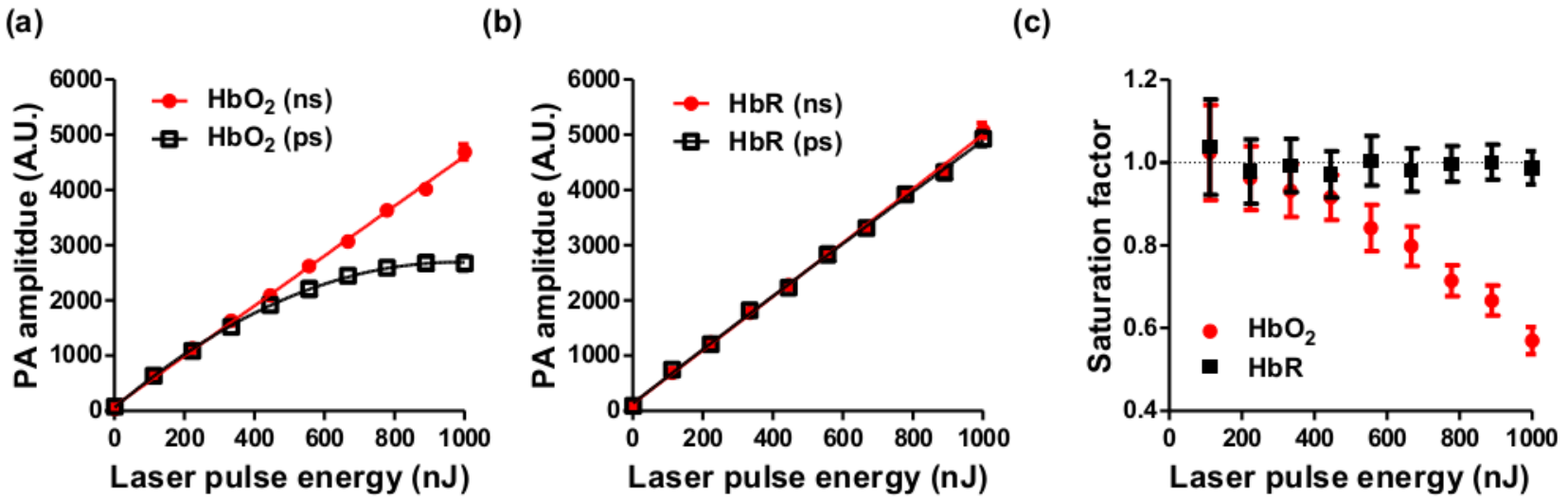
Saturable absorption from Wikipedia



„At sufficiently high incident light intensity, atoms in the ground state become excited into an upper energy state at such a rate that there is insufficient time for them to decay back to the ground state before the ground state becomes depleted, and the absorption subsequently saturates“

https://readingpenrose.files.wordpress.com/2014/04/absorption-1.gif

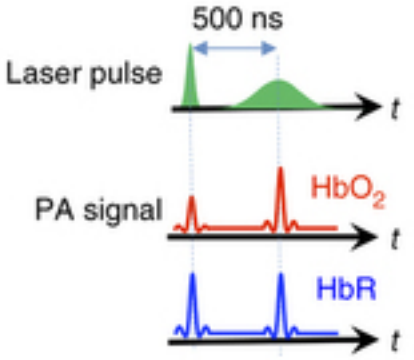
Absorption saturation of oxy- and deoxy-hemoglobin (HbO_2 and HbR)



$$\text{Intensity}\left[\frac{\text{Watts}}{\text{cm}^2}\right] = \frac{\text{Laser peak power [W]}}{\text{Effective focal spot area [cm}^2\text{]}}$$

while the peak power is defined as

$$\text{Peak power [W]} = \frac{\text{Laser pulse energy [J]}}{\text{Pulse duration [seconds]}}$$

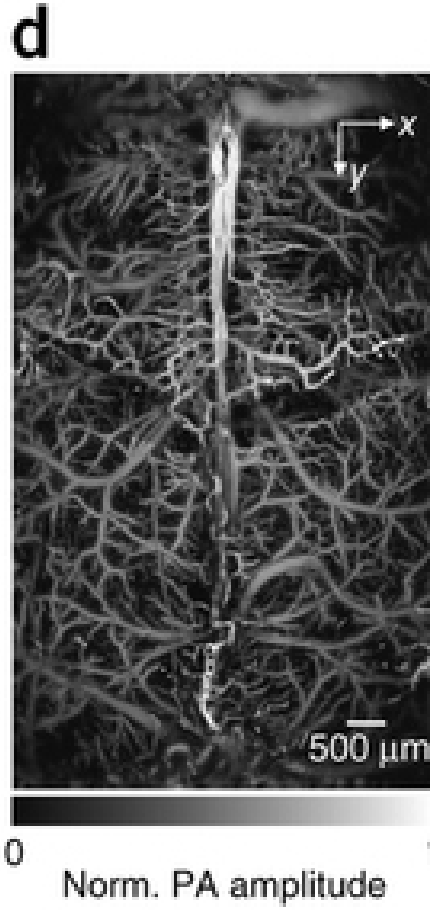


Single-wavelength pulse-width-based measurement of O_2 saturation (PW-sO_2)

http://lidaris.com/glossary-2/fluence/

Structural capabilities of the system

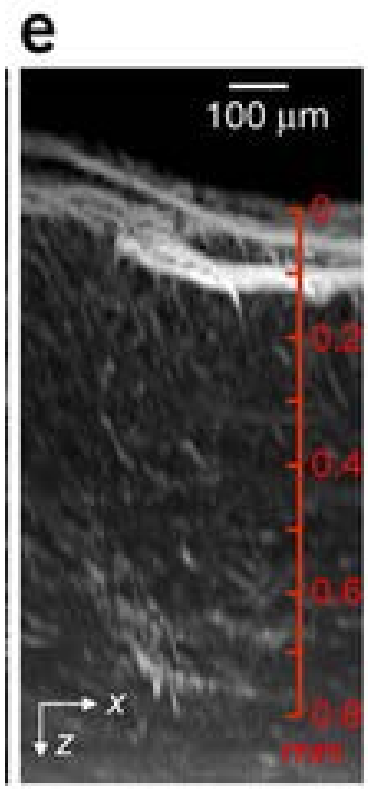
5 × 10 mm^2 region of the mouse brain through intact skull with the scalp removed. optical focal plane ~250 μm beneath skull surface

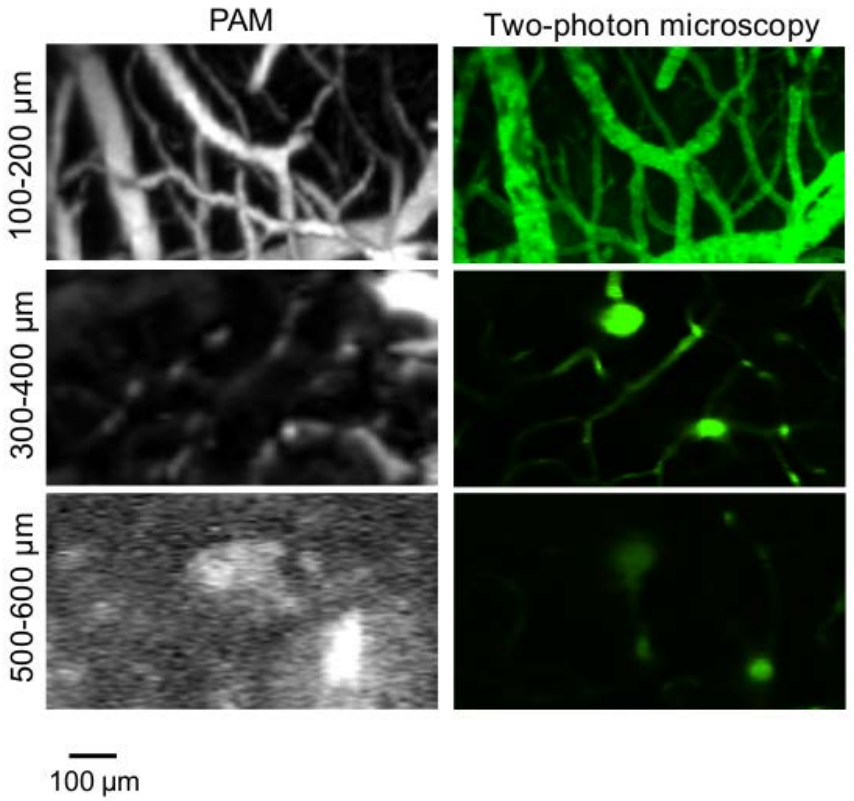


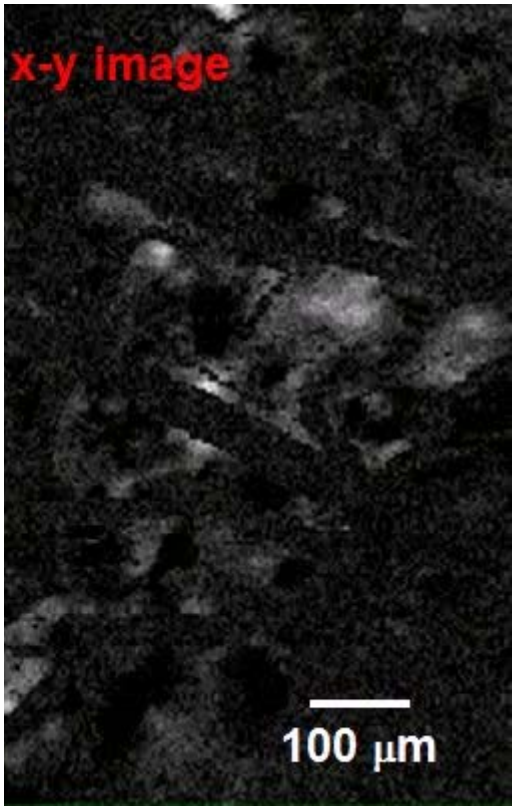
(acquisition time: ~15 s).

Structural capabilities of the system

depth-scanning of the optical focal zone with a z-step size of 100 µm,
imaging depth of ~0.7 mm possible
= effective pixel count of ~47 in focus along the depth direction.





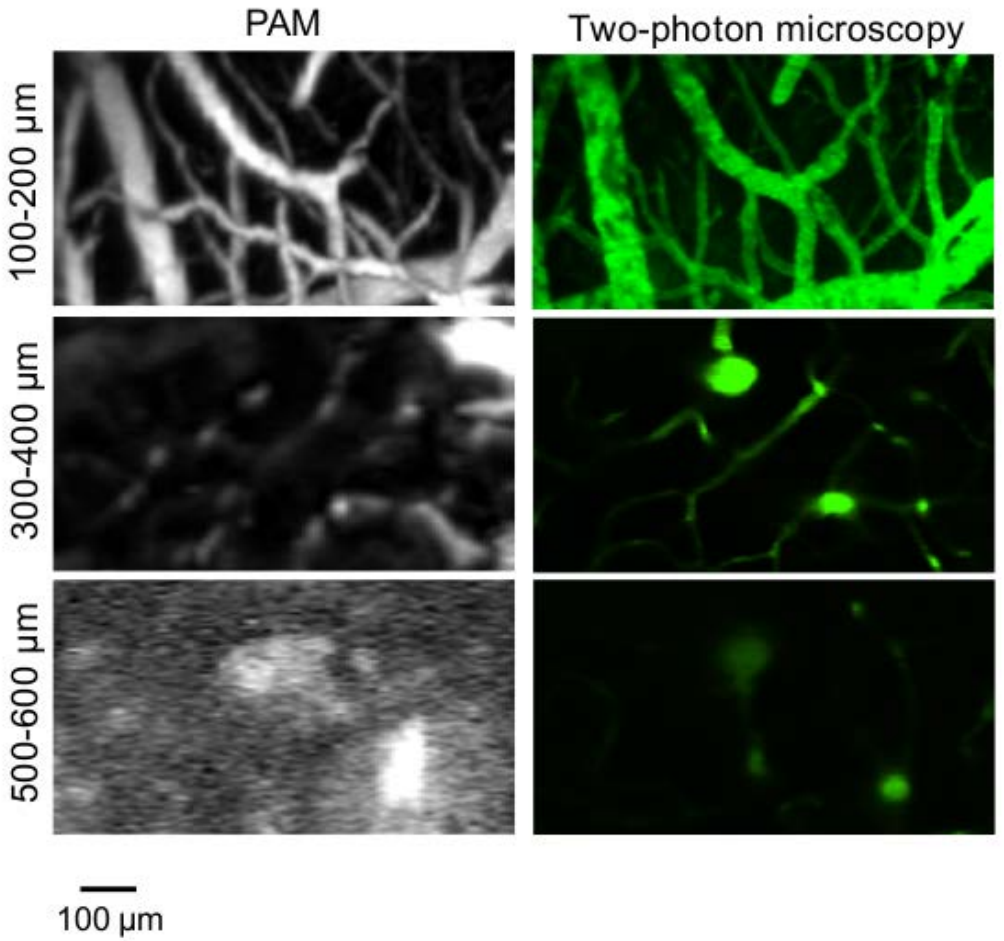


Imaging of deep capillaries not possible!

Structural capabilities of the system

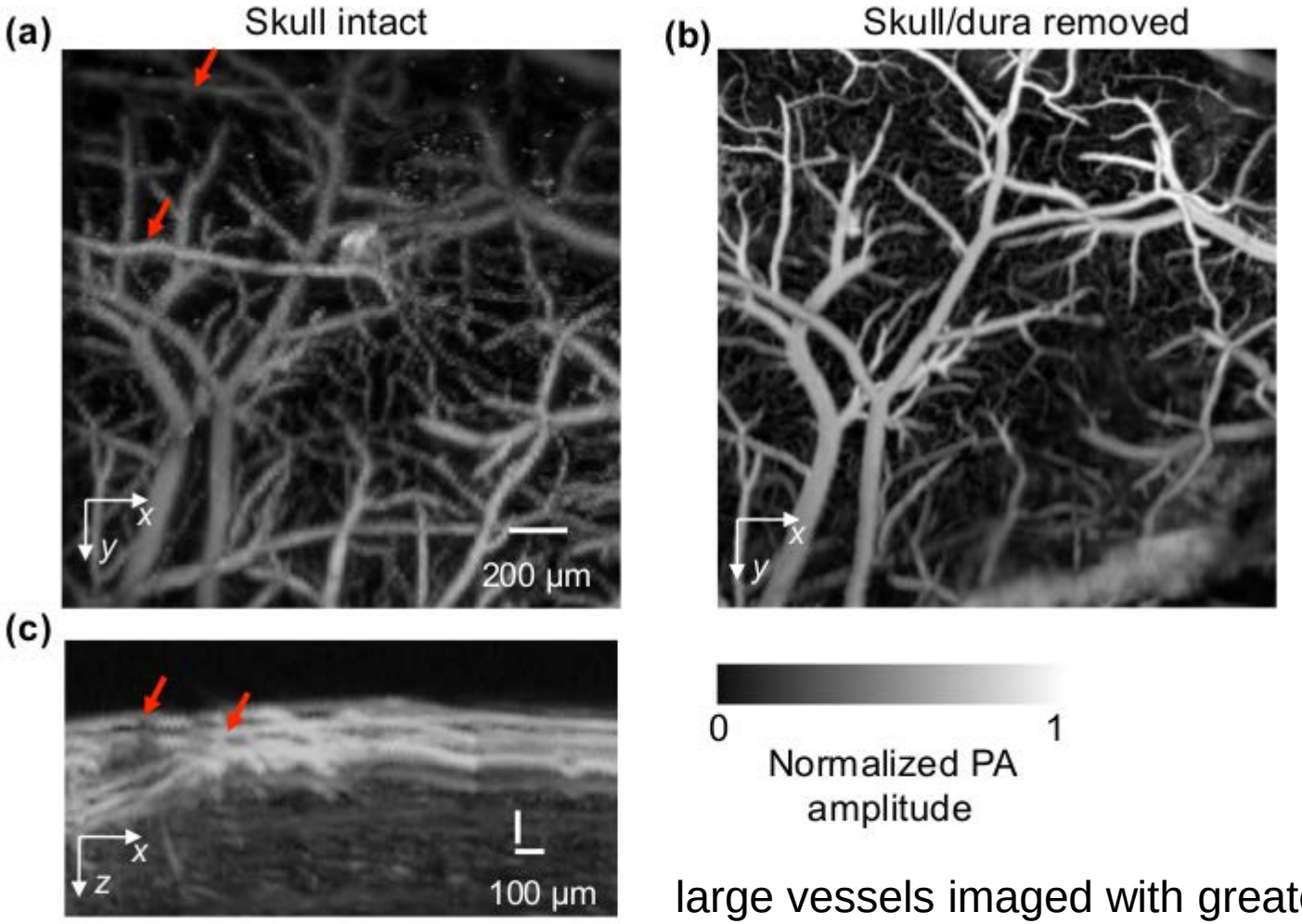
Comparison to TPM

Pia-vessels



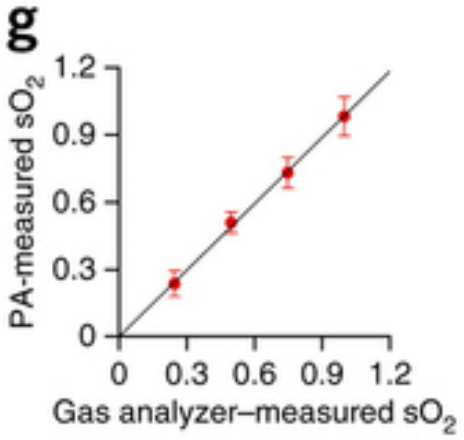
Contrast: FITC-labelled dextran

Effect of the skull



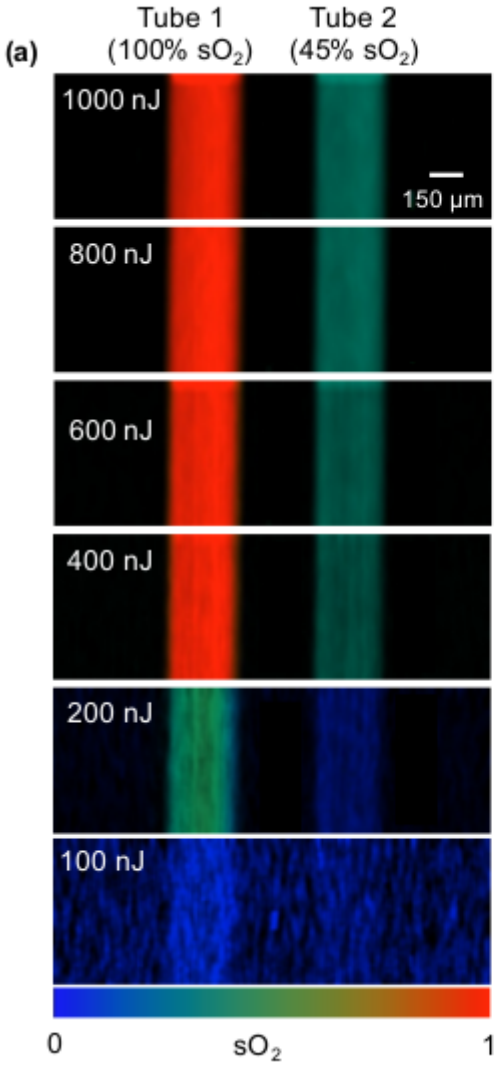
large vessels imaged with greater SNR, more deep capillaries imaged, but spatial resolution only marginally improved

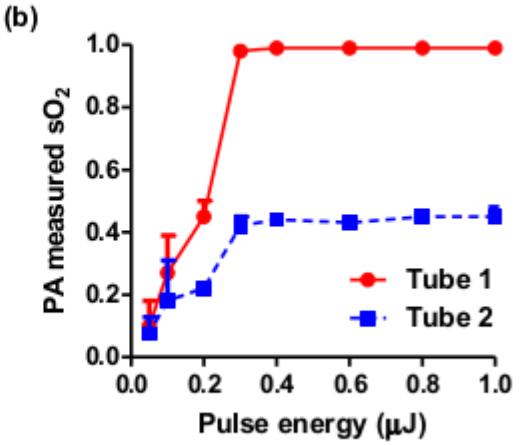
Calibration of O_2 measurements



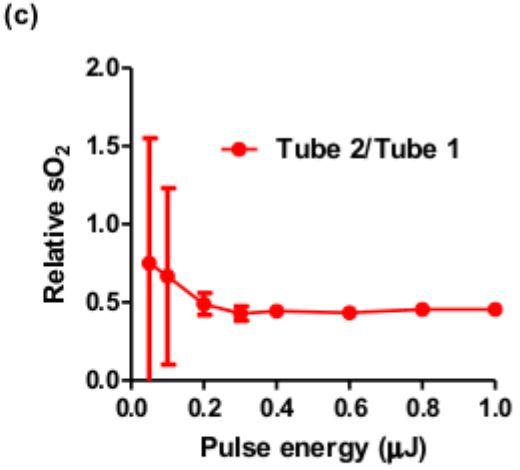
Comparison to blood phantoms

- Average measurement error (s.e.m.) ~2.7%



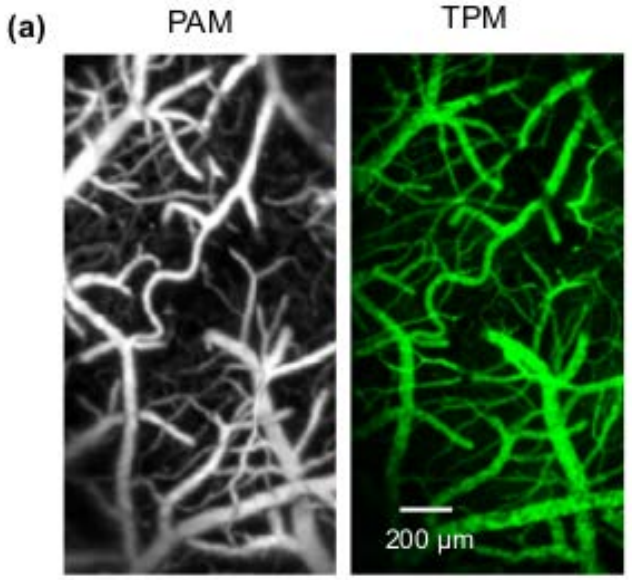


error <3% with pulse energies ≥300 nJ



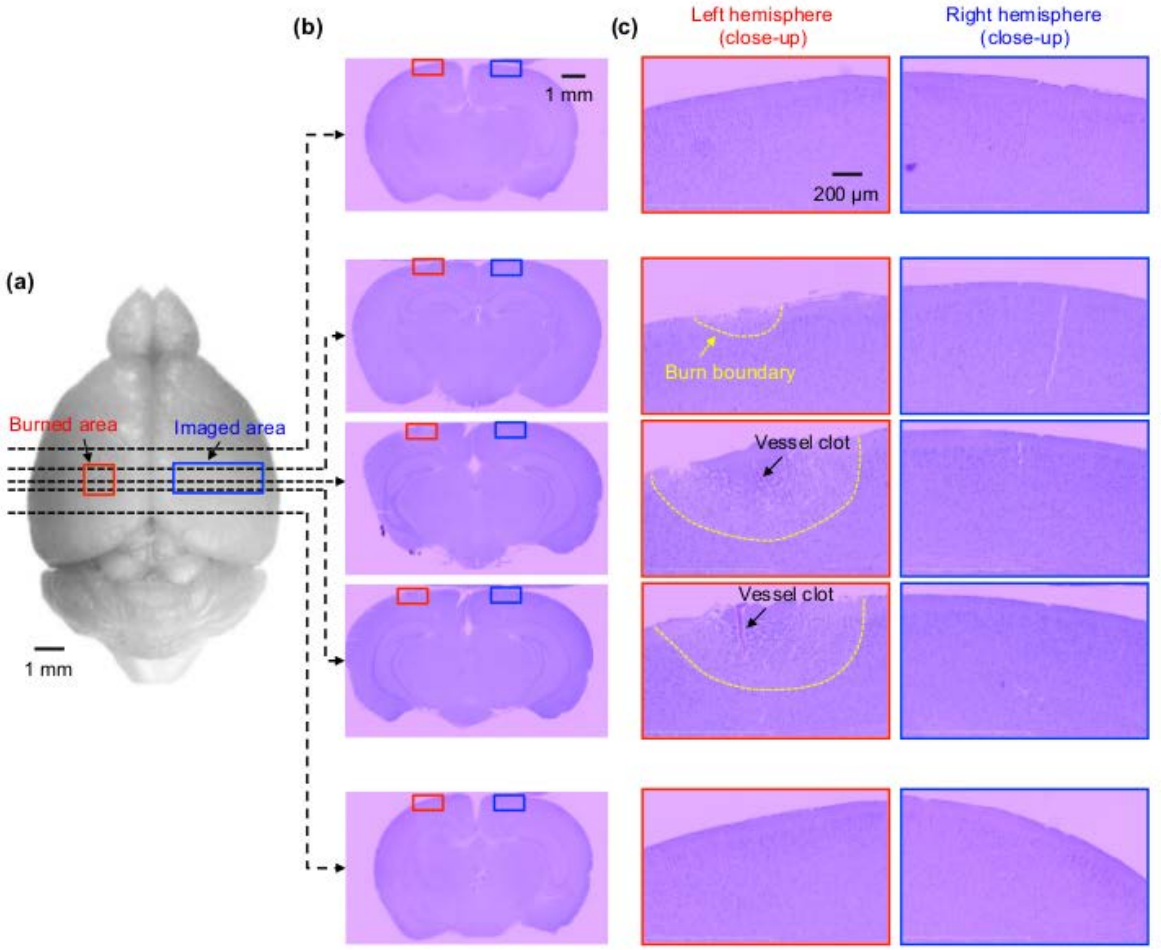
error <2% with pulse energies ≥300 nJ

Assessment of toxicity



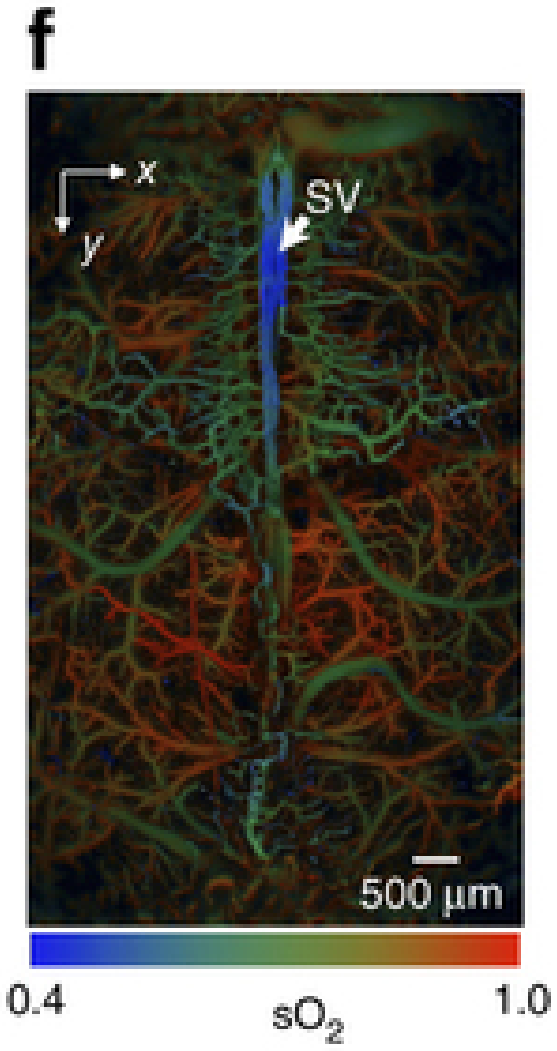
TPM with FITC

- No leakage



no burn damage

O2 measurements in rest

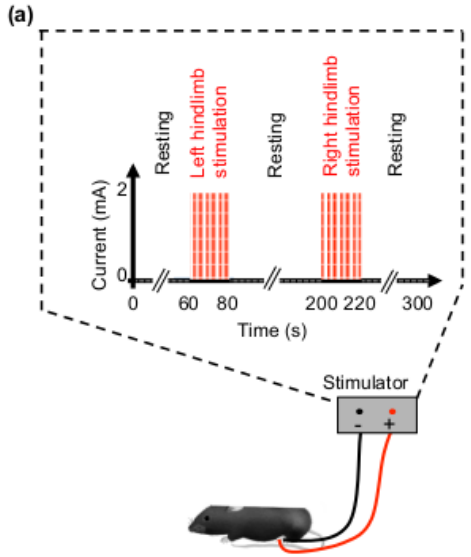


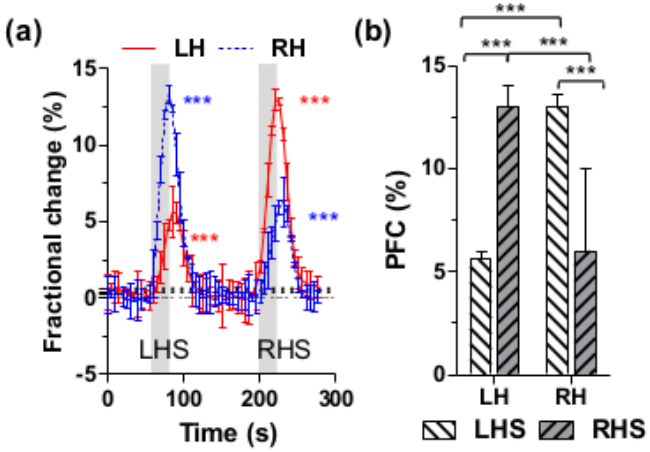
- Mapping of sO_2 of the mouse brain vessels using
- PW-sO_2
- acquisition time: ~40 s
- pulse energy: 400 nJ
- nonsaturated PA signal to correct for optical attenuation and the laser spot size

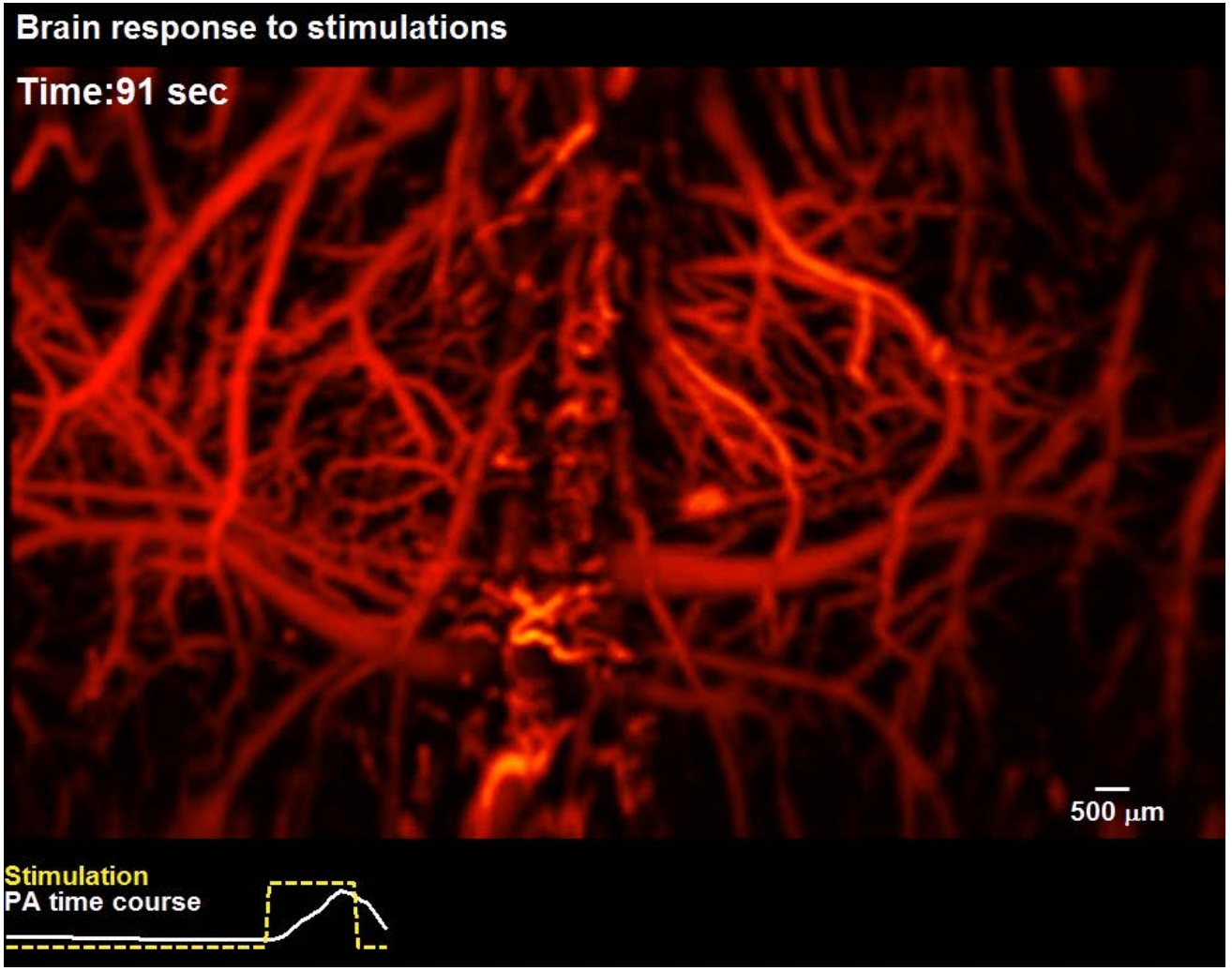
„The averaged sO 2 level observed in the skull vessels was lower than that in the cortical vessels, a result consistent with the low-oxygenation microenvironment in
bone marrow.“

(SV = skull vessel)

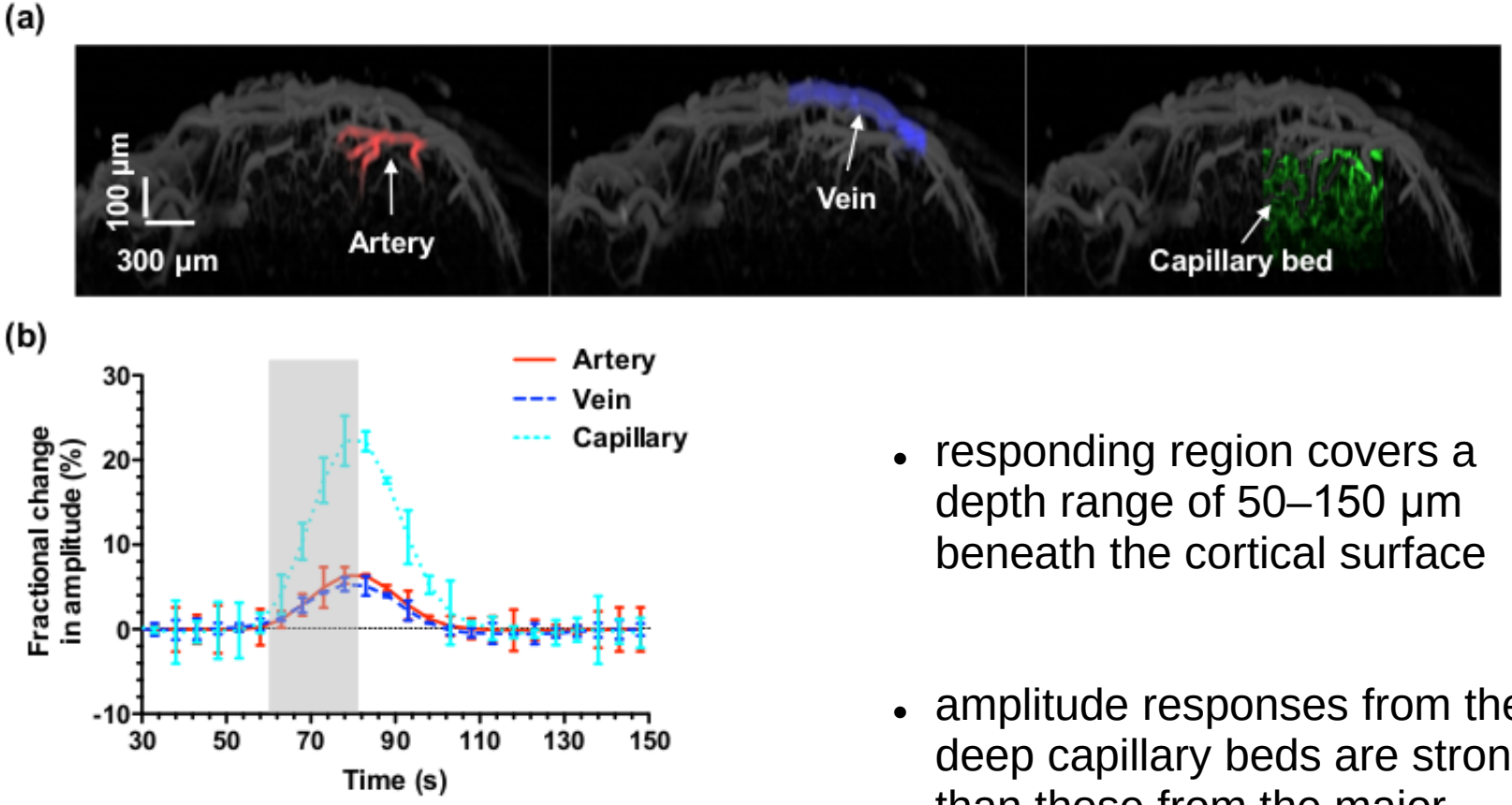
O2 measurements in somatosensory cortex upon sensory stimulation





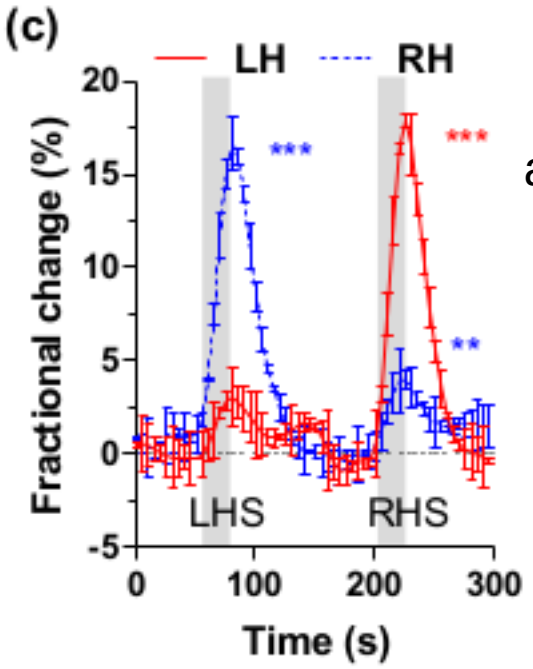


Analysis of depth

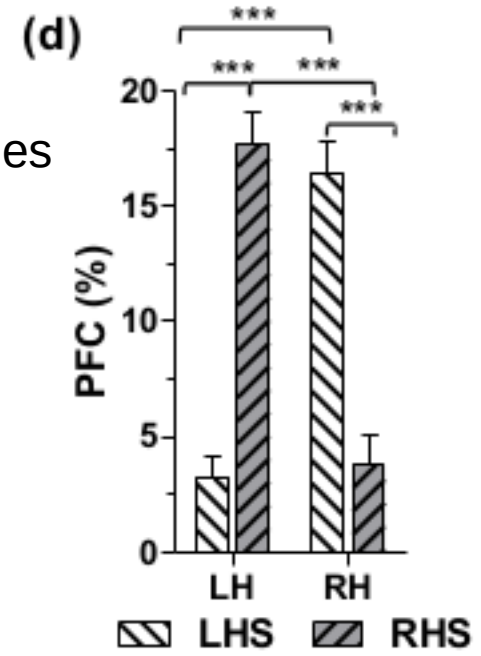


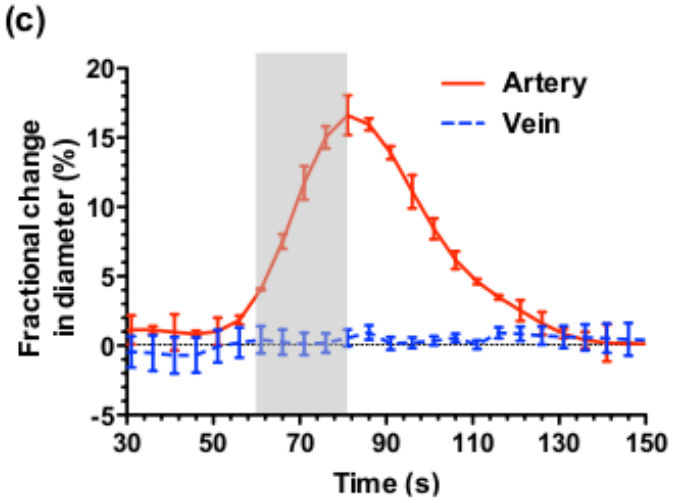
- responding region covers a depth range of 50–150 µm beneath the cortical surface
- amplitude responses from the deep capillary beds are stronger than those from the major arteries and veins

Analysis of vessel dilatation



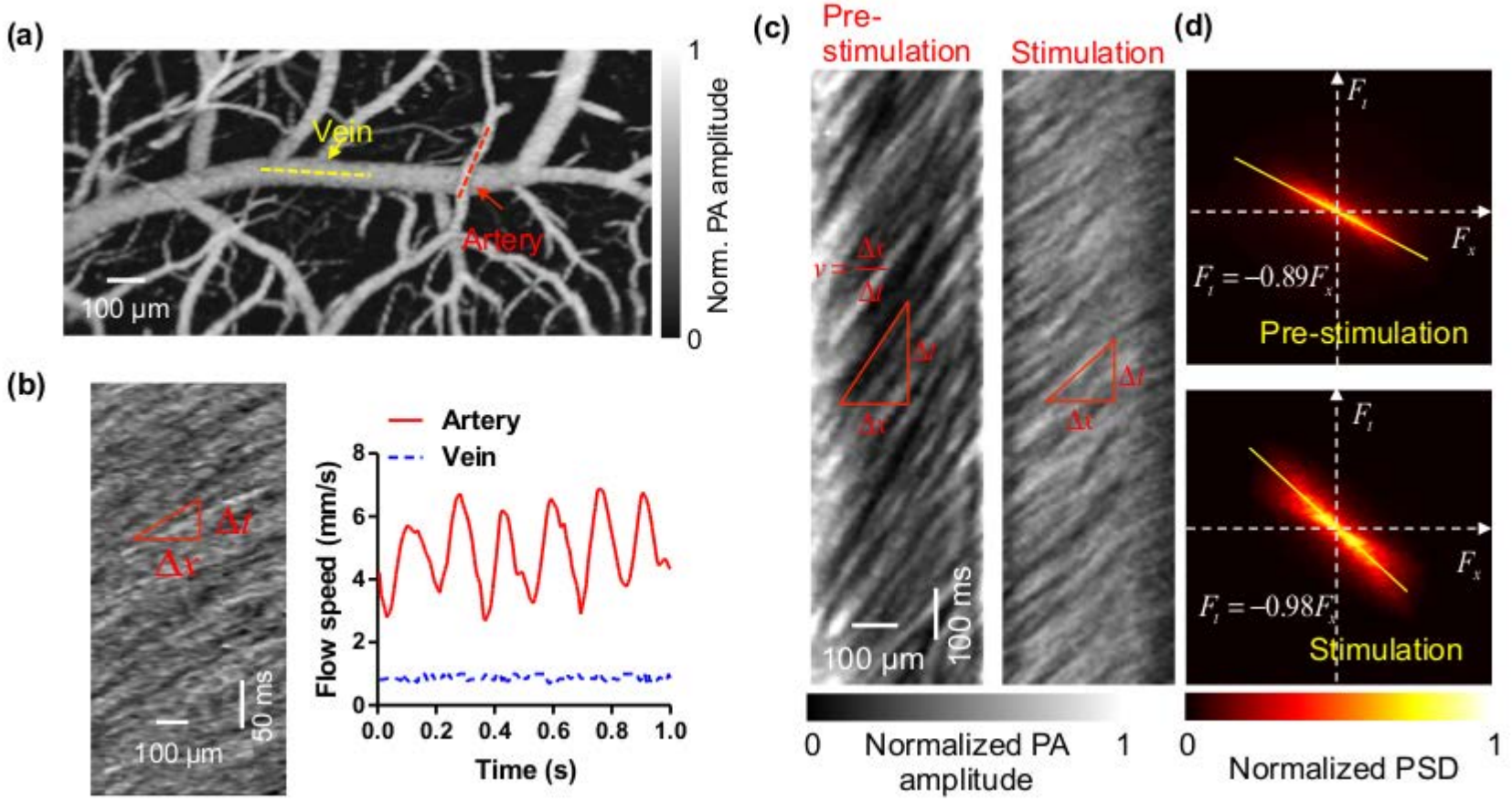
arteries



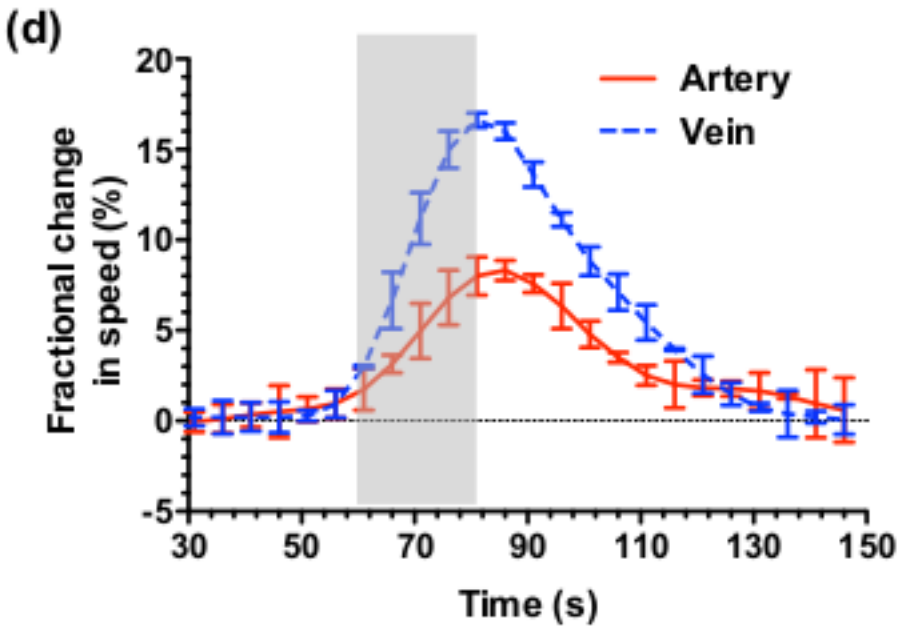


- Capillary diameter changes were not resolvable
- the artery dilated substantially in the contralateral hemisphere
- ipsilateral arterial dilation was also observed but with a much weaker magnitude
- Veins did not show dilations

Analysis of blood flow speed by fast line scanning

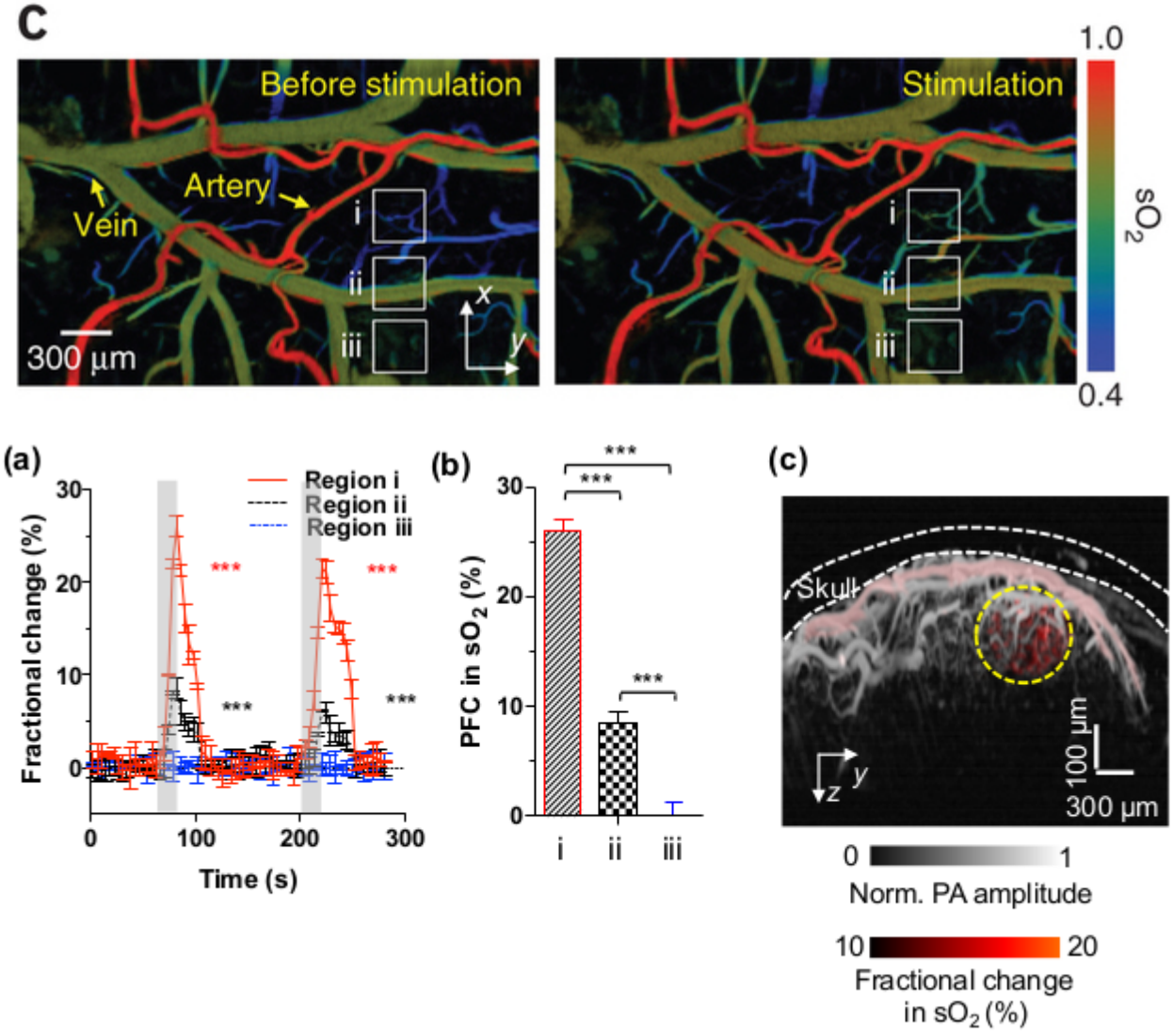


Analysis of blood flow speed by fast line scanning

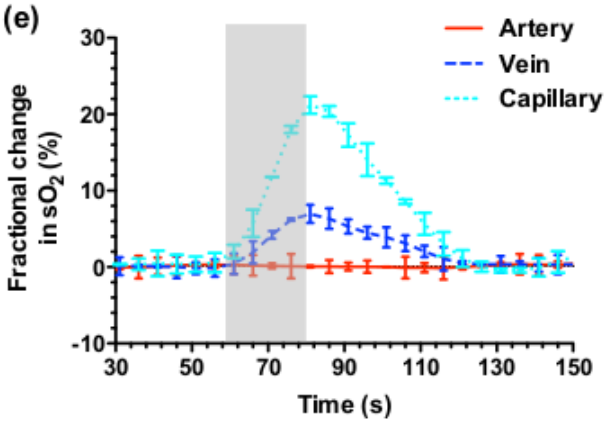


Stimulations induced a substantial increase in blood flow speed in both arteries and veins

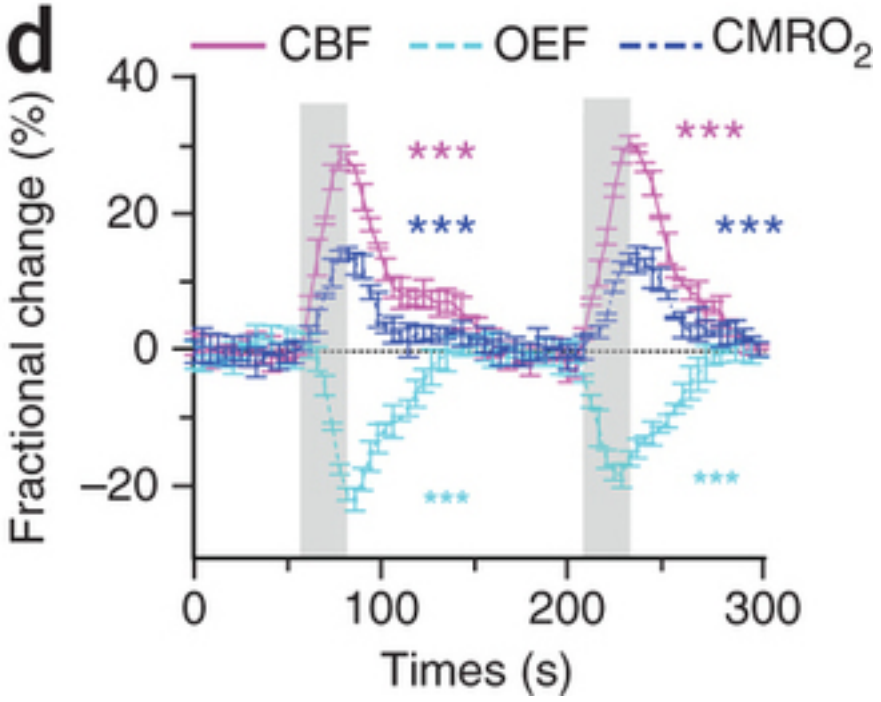
Analysis of sO_2 levels in subregions



- The fractional change in sO 2 diminished with increasing distance from the core responding region
- which was ~100 µm below the cortical surface
- sO 2 increase was greater in deep capillary beds than in veins and was insignificant in arteries



Estimation of cerebral oxygen metabolism



CBF: cerebral blood flow
OEF:
$CMRO_2$:

- moderate fractional increase in $CMRO_2$, peaking at ~15%
- ratio between fractional changes in CBF and $CMRO_2$ (i.e., the flow-consumption ratio) was ~2.0

Comparison of the methods

	PAT	TPM
Preparation of the animal	Removal of skin	Cranial window, injection
xy-resolution		
z-resolution		
penetration depth		
speed		
Oxygen level in vessel		
Oxygen level in parenchyma		
costs		

Thank you!