HyperProbe

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Hyperspectral Characterization of Hemodynamic and Metabolic Responses in Mouse Brain During Oxygenation Alterations

Pietro Ricci[a,b,*], Emilia Conti, Charly Caredda, Ivan Ezhov, Luca Giannoni, Dorotea Nardini, Anam Toaha, Jessica Lucchesi, Bruno Montcel, Daniel Rueckert, Ilias Tachtsidis, Francesco S. Pavone

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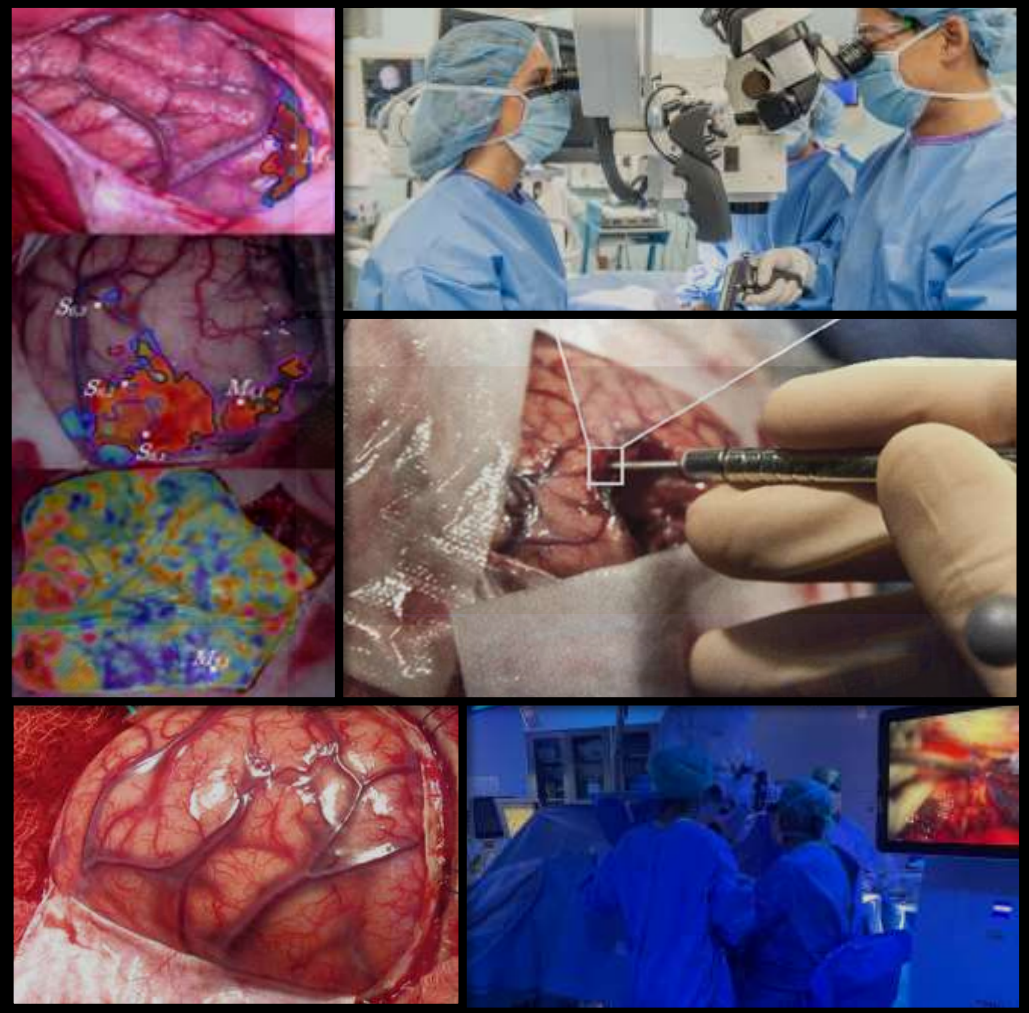
LENS European Laboratory for Non-Linear Spectroscopy

Funded by the European Union



- Monitoring the physiological state of the **exposed brain surface** remains a key challenge in neuroscience and **neurosurgery**
- **Local oxygen availability** varies over time, affecting perfusion, vascular oxygenation, and cellular metabolism
- Current techniques often provide **incomplete** or **indirect** information, with **limited** spatial coverage or molecular specificity

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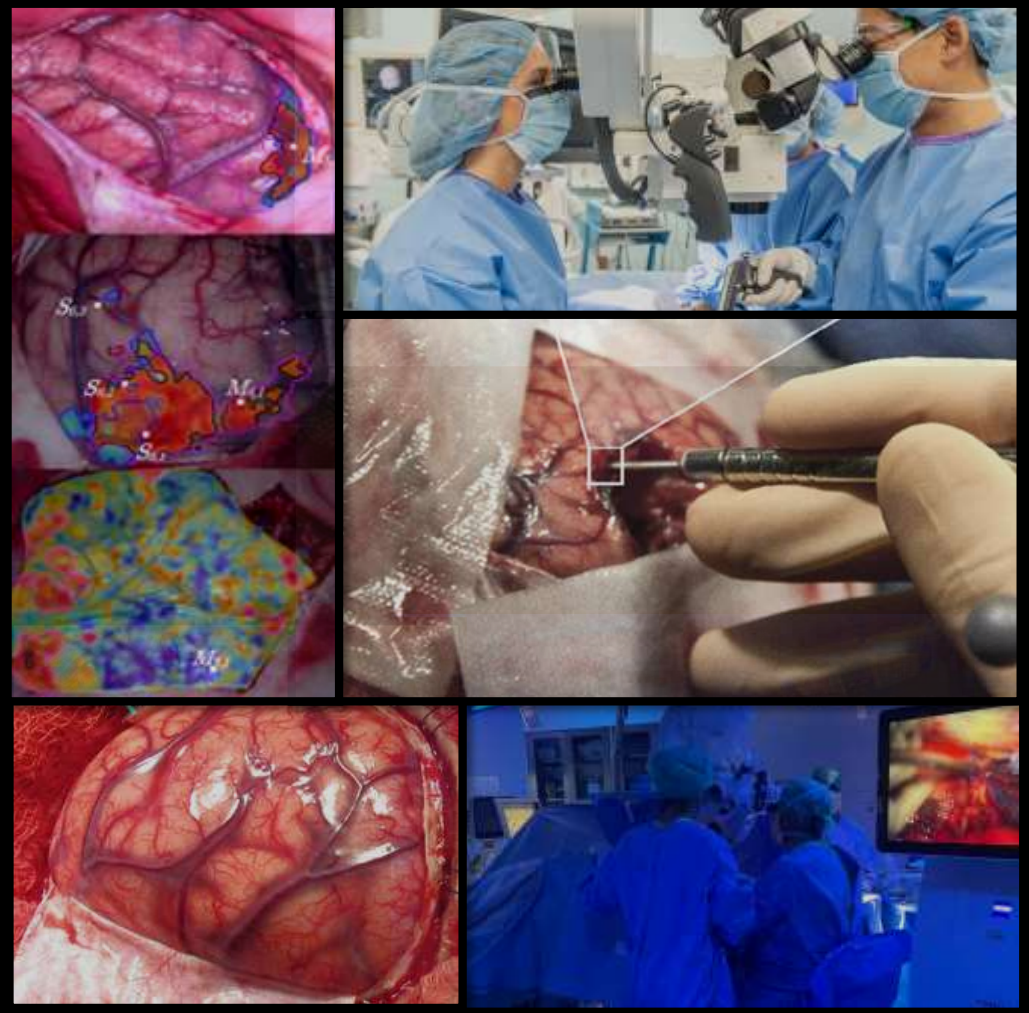

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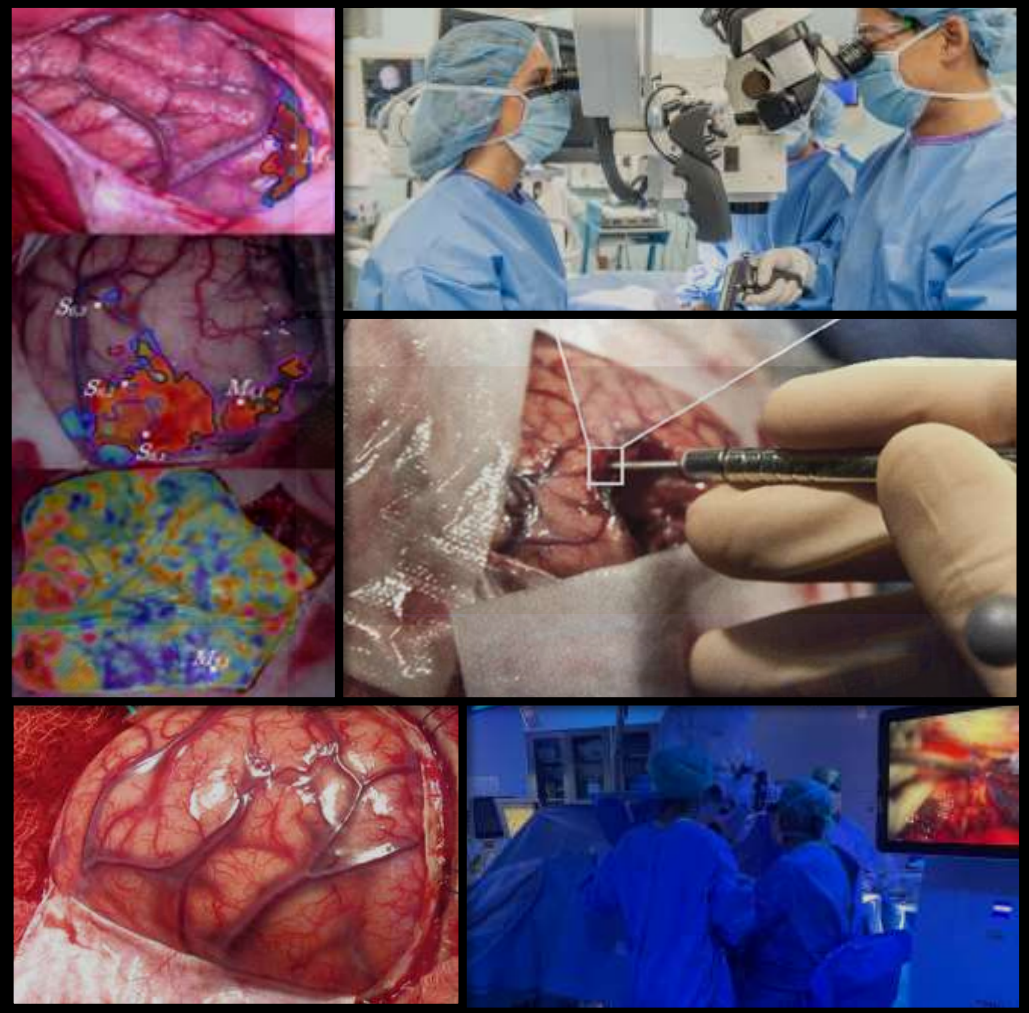

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Overcoming current limitations requires the development of **new, physiologically and molecularly** informed **approaches** to brain monitoring

Signal dynamics must be understood in the presence of **intact perfusion, vascular regulation, and metabolic demand**

In vivo preclinical validation is essential and provides a **controlled**, robust framework to investigate these processes

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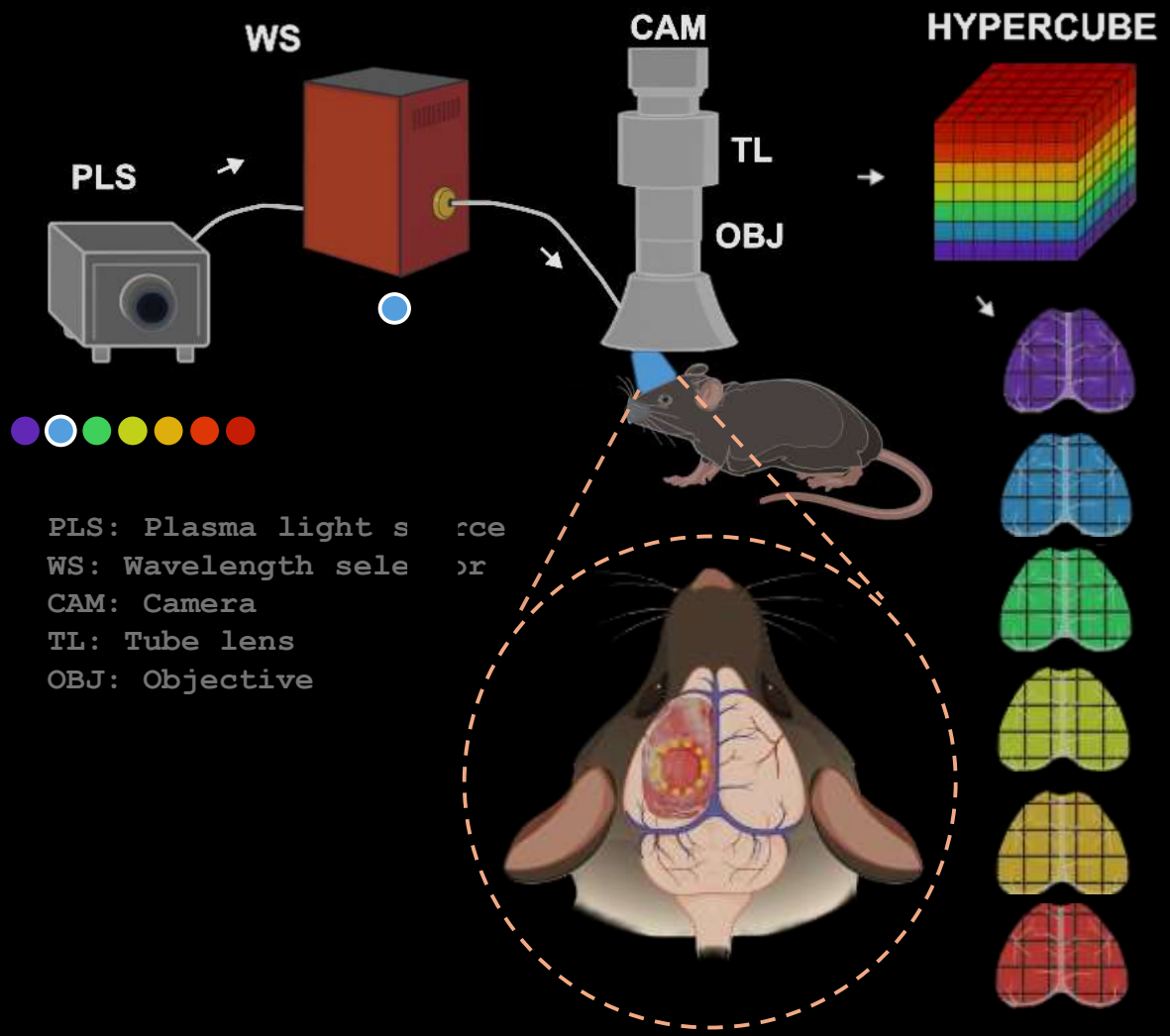
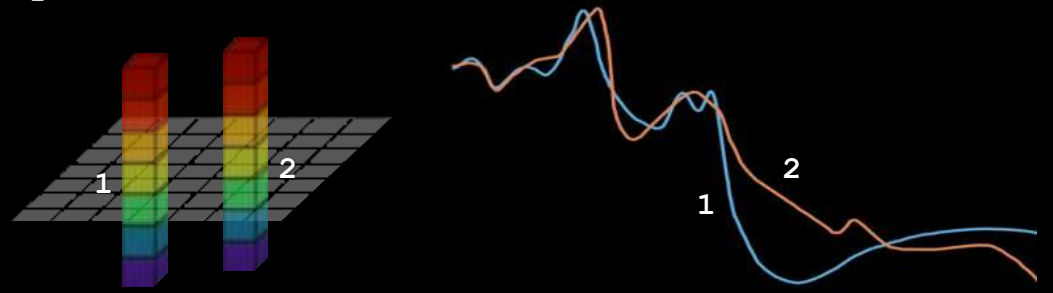
Hyperspectral Characterization of Hemodynamic and Metabolic Responses in Mouse Brain During Oxygenation Alterations

In vivo Hyperspectral imaging



Hyperspectral imaging captures 2D **reflectance** images under illumination at contiguous wavelengths, producing a 3D spatio-spectral data cube



This representation enables the extraction of a reflectance spectrum at the **single-pixel level**

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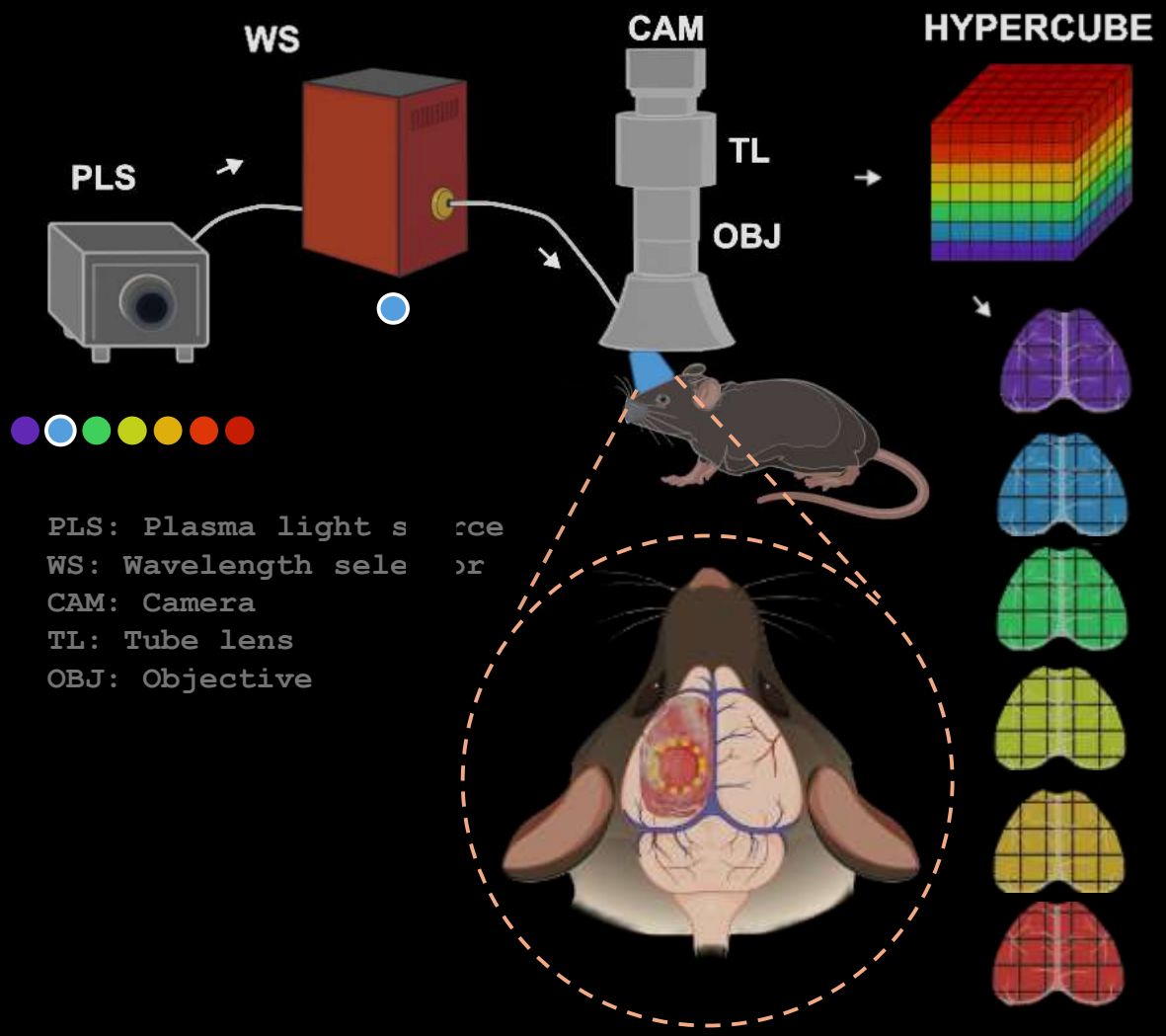
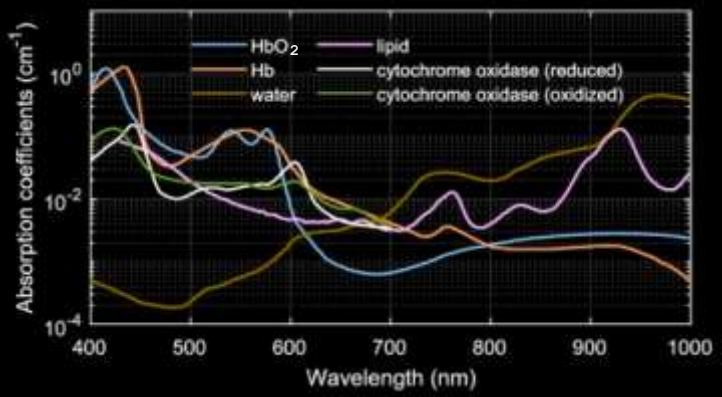
In vivo Hyperspectral imaging

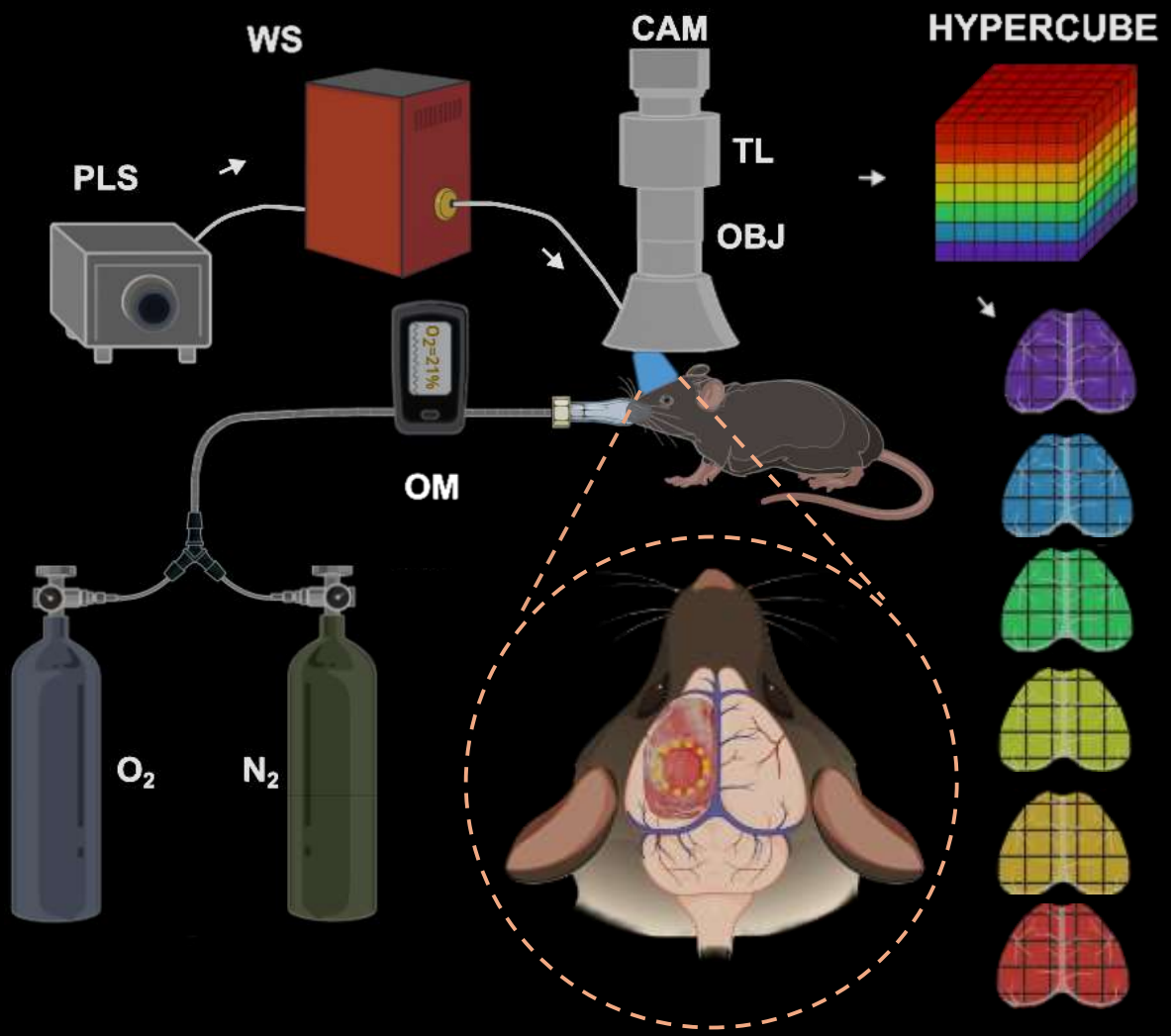


By measuring the reflected light from biological tissue, **molecular compounds** are directly **inferred, imaged and quantified**

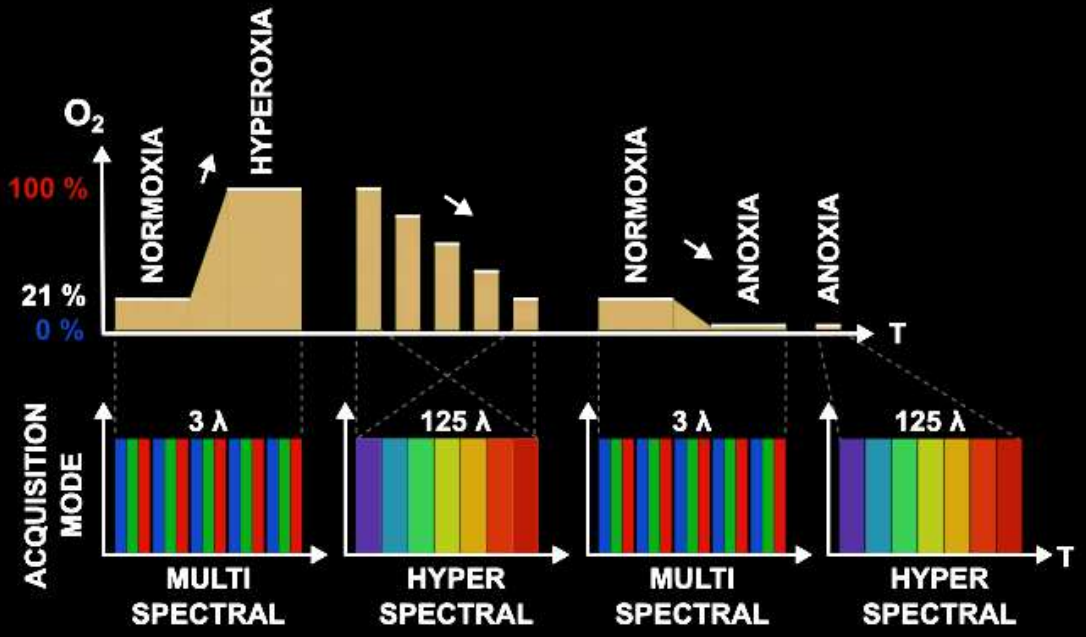


By **spectral unmixing**, different molecular contributions and their relative change in time and space are disentangled

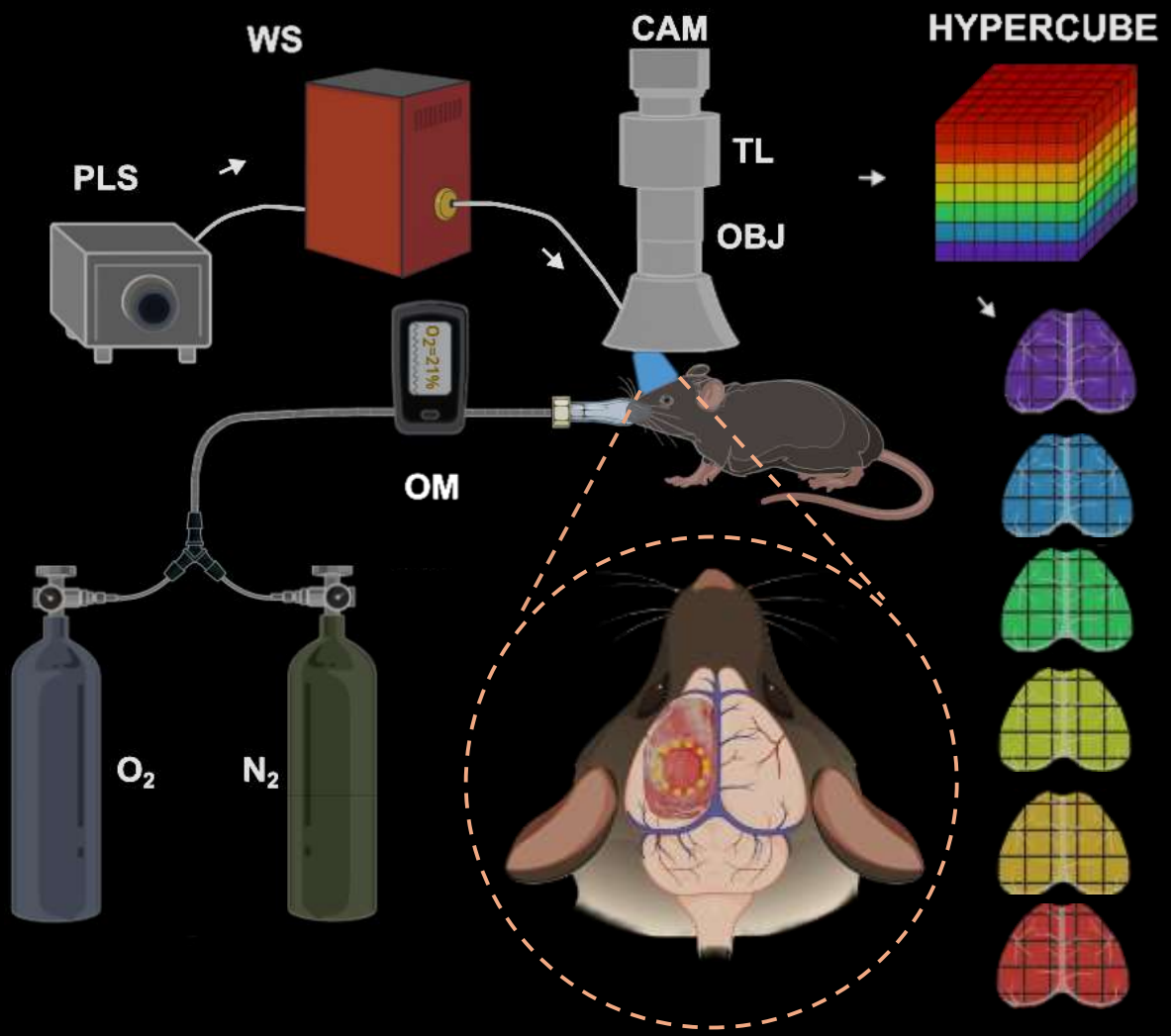
In vivo Hyperspectral imaging



Cerebral **hemodynamics** and **metabolism** are characterized while evolving under varying oxygenation conditions



In vivo Hyperspectral imaging



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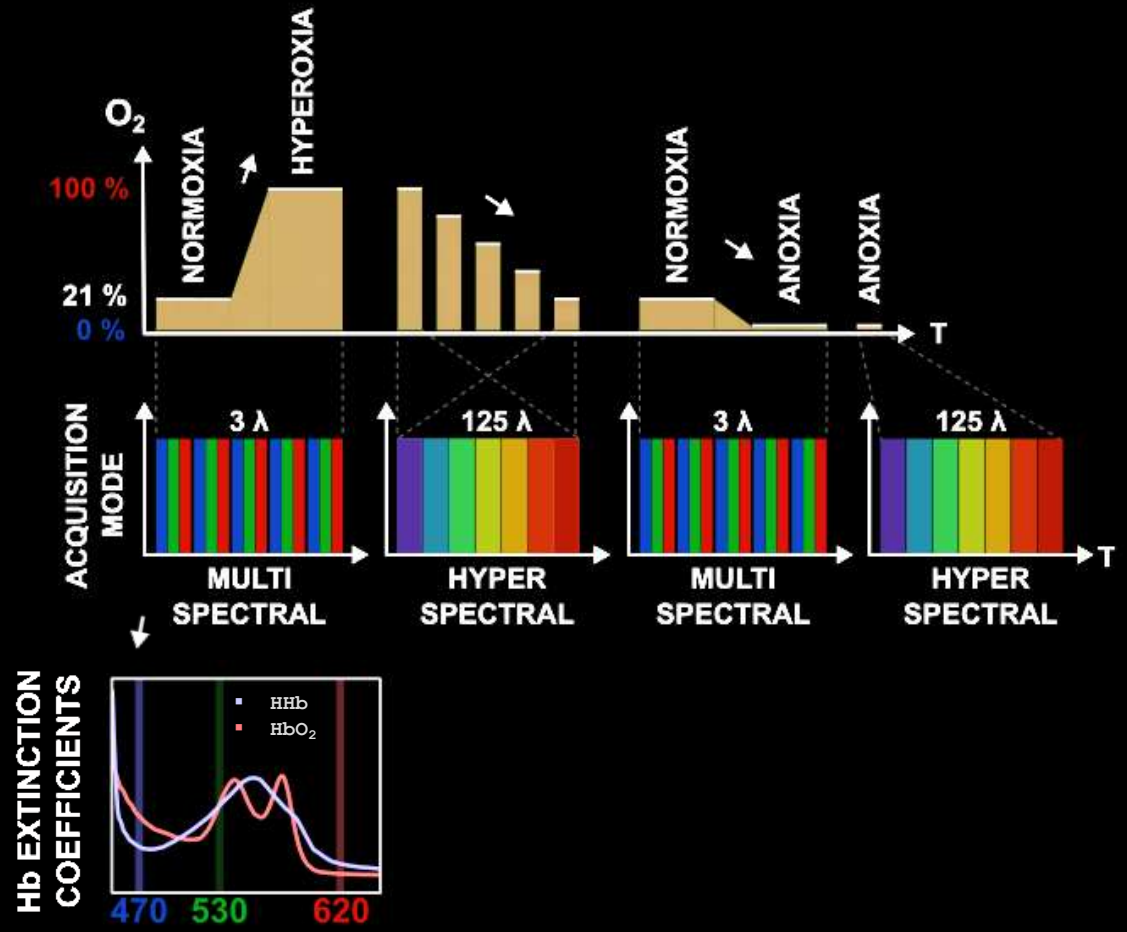
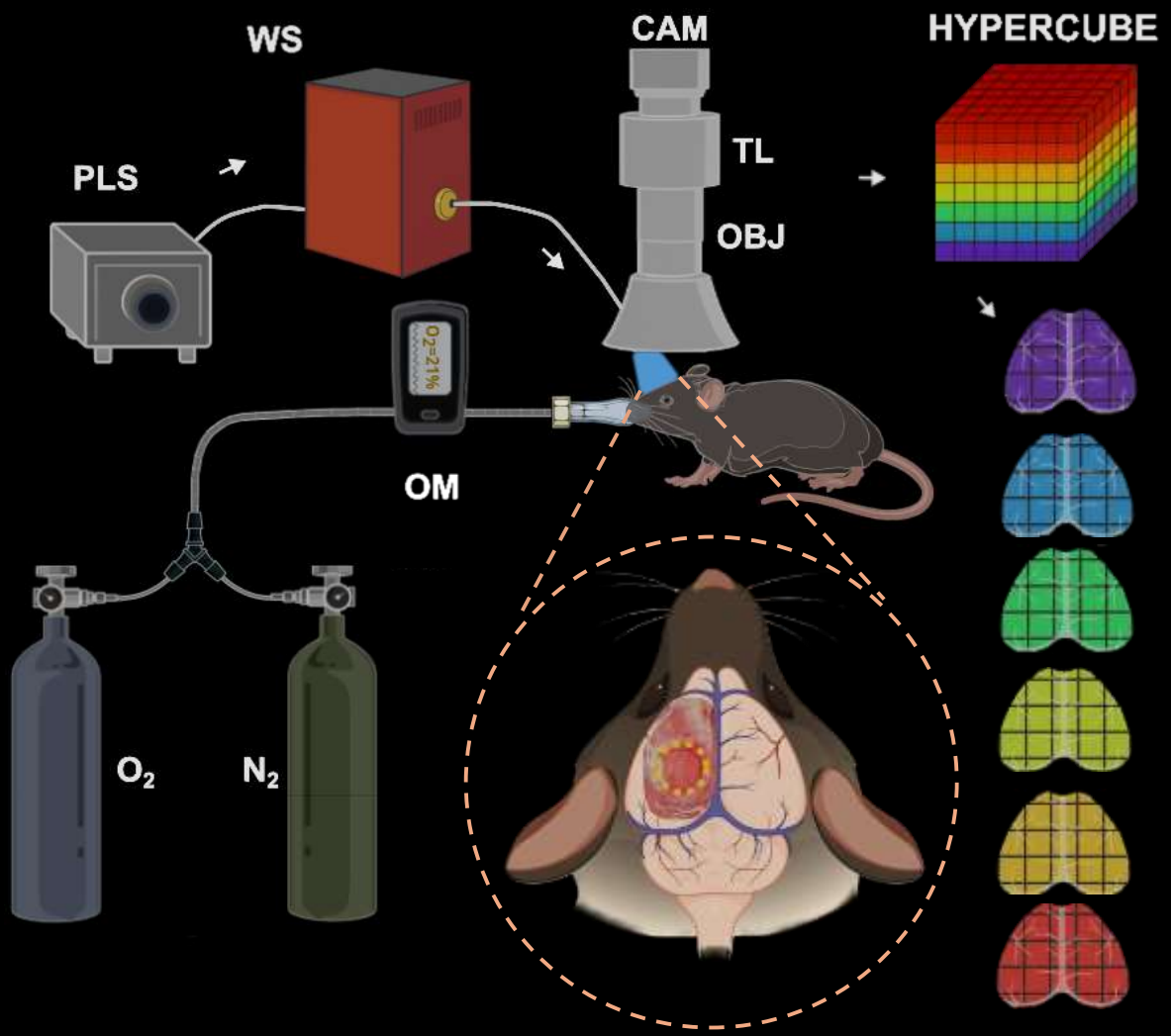


In vivo Hyperspectral imaging



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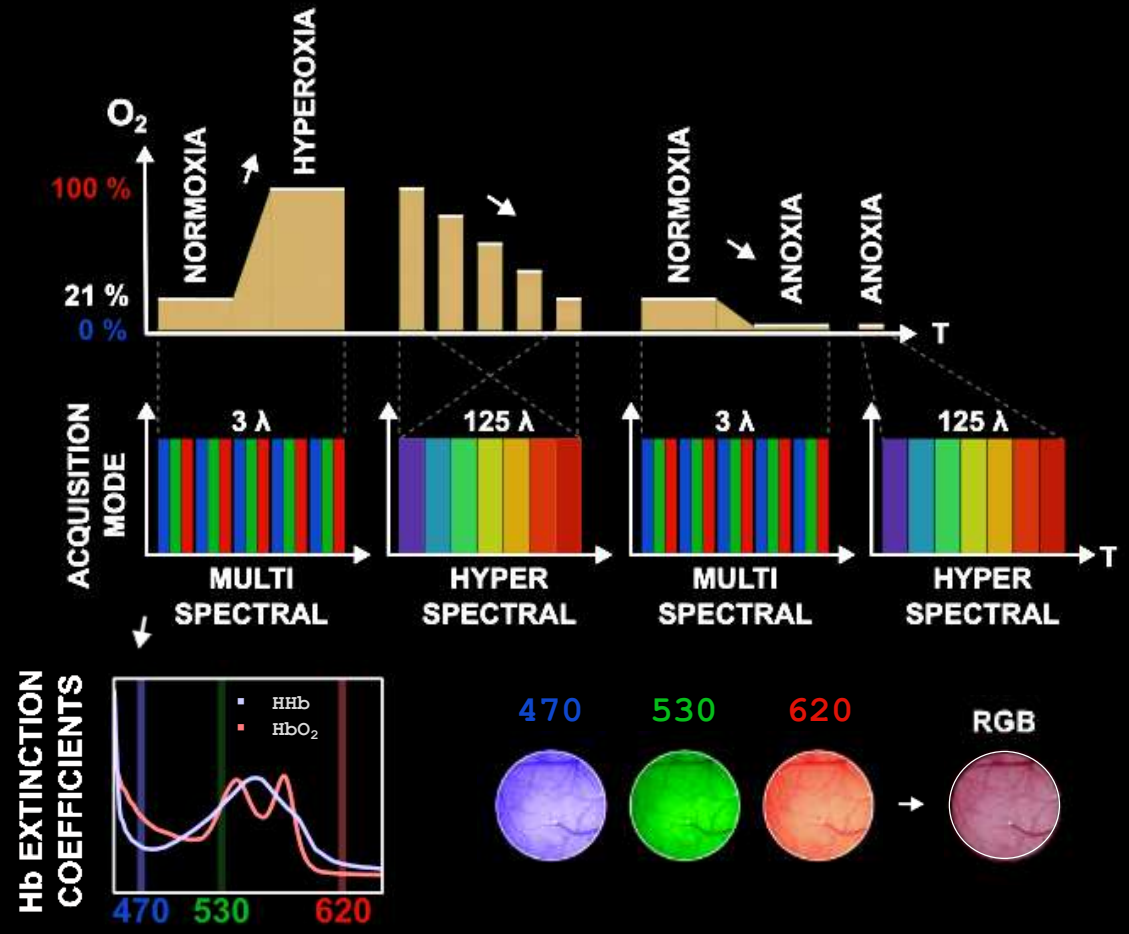
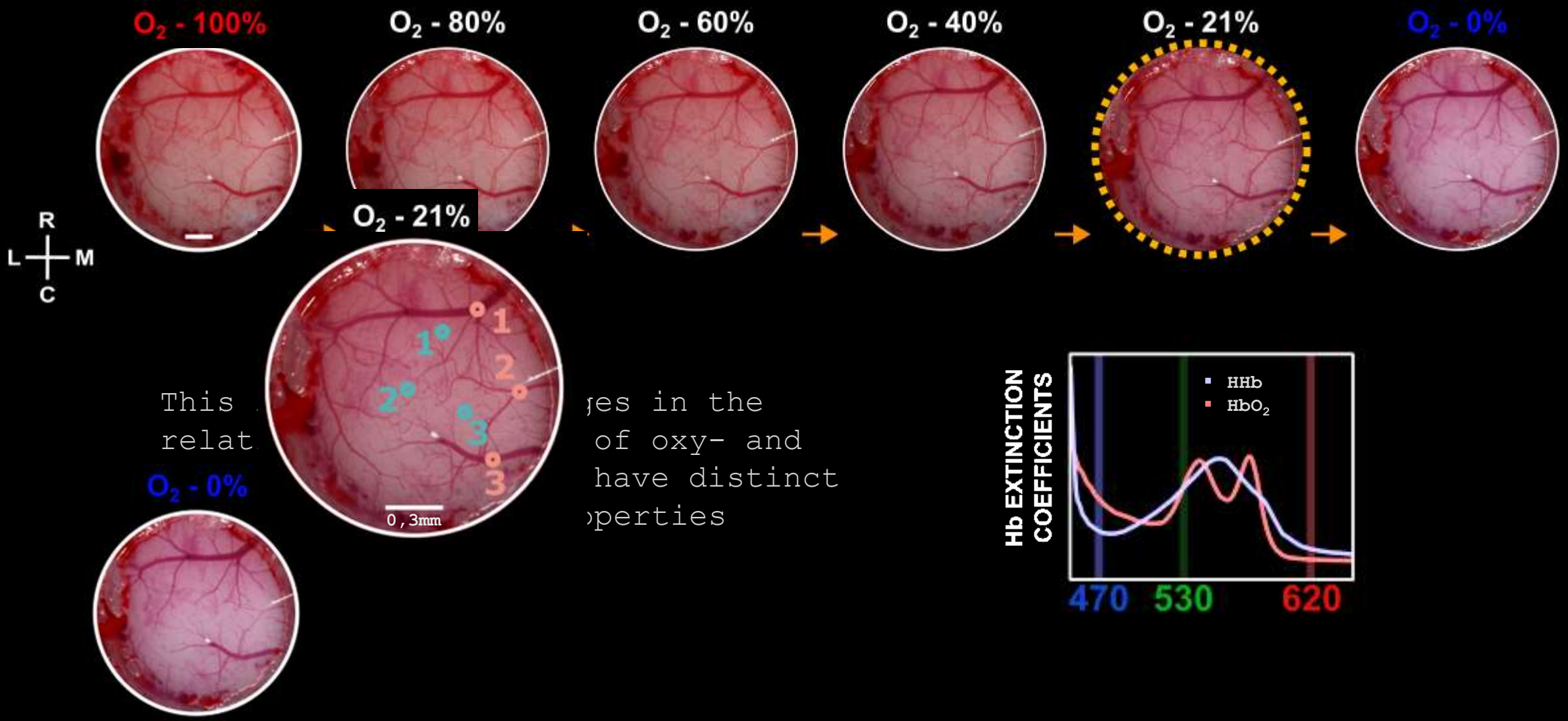


Clear oxygenation-dependent changes in cortical appearance observed in RGB images



This
relat
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of oxy- and
have distinct
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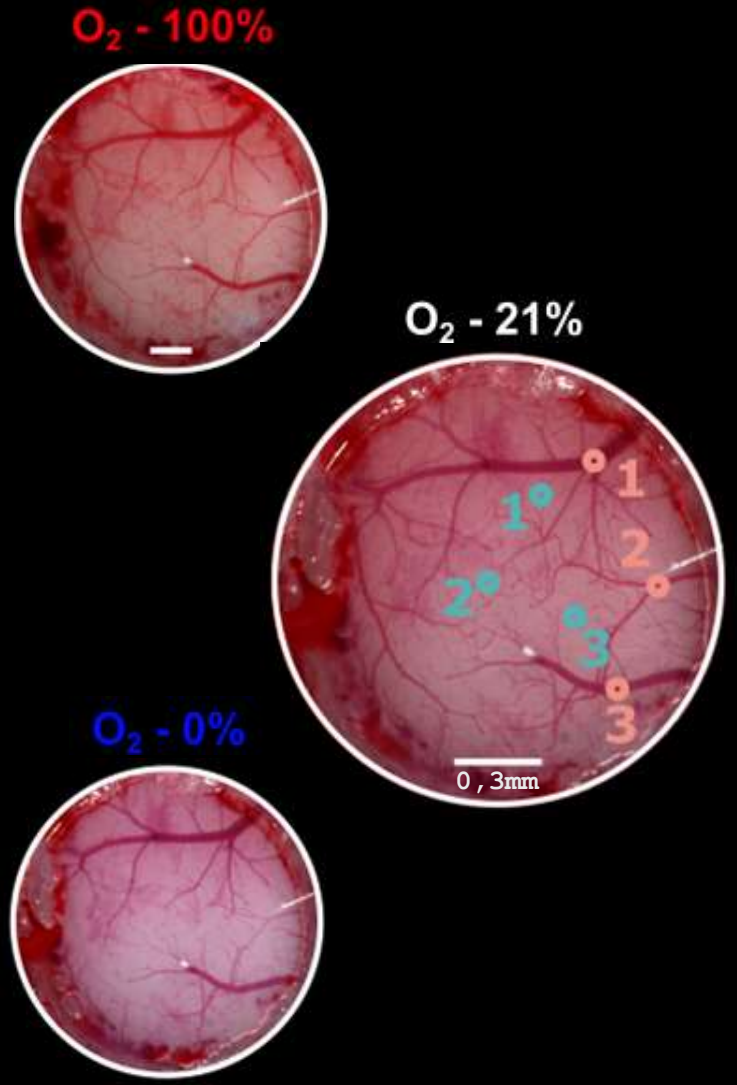
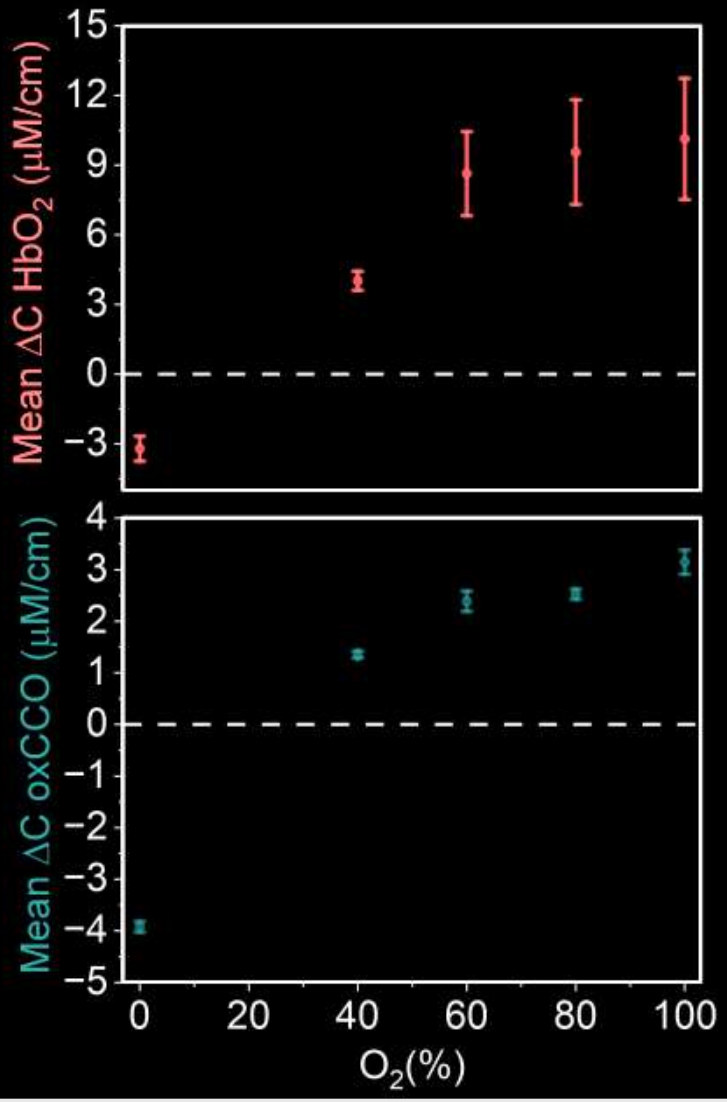
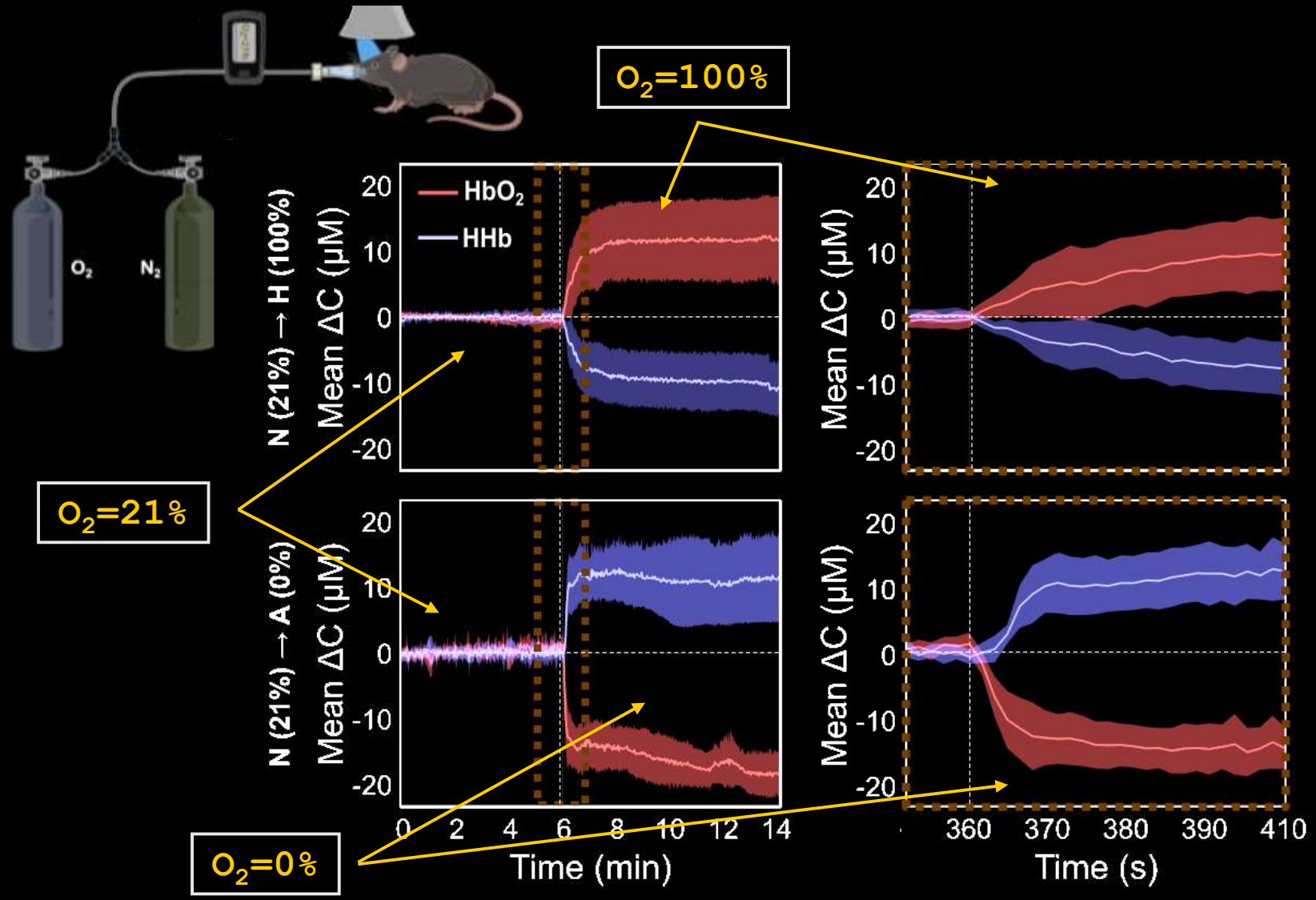
We quantify molecular changes, including **hemoglobin** species and the redox state of **mitochondrial cytochrome c oxidase**





- HbO_2 systematic variation with inspired oxygen fraction reflect strong modulation in **vascular regions**
- oxCCO changes in **extravascular tissue** indicate modulation of mitochondrial metabolism

Multispectral imaging enabled **continuous** monitoring of oxygenation transitions, enabling quantification of HbO_2 and HHb changes relative to a normoxic baseline



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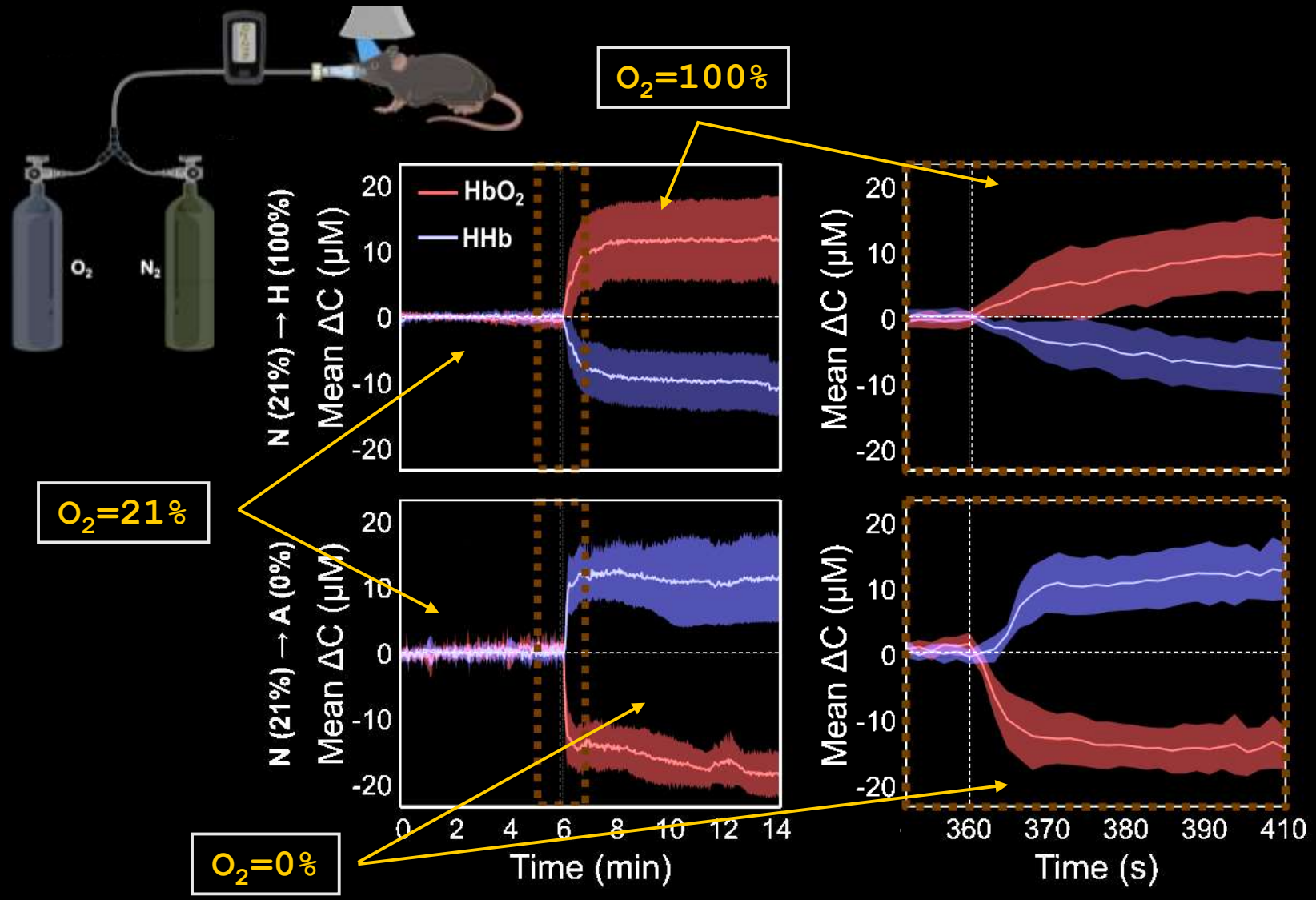

- **Rapid** hemodynamic changes following oxygen modulation
- HbO_2 and HHb show **complementary** temporal dynamics in **anoxia** and **hyperoxia**

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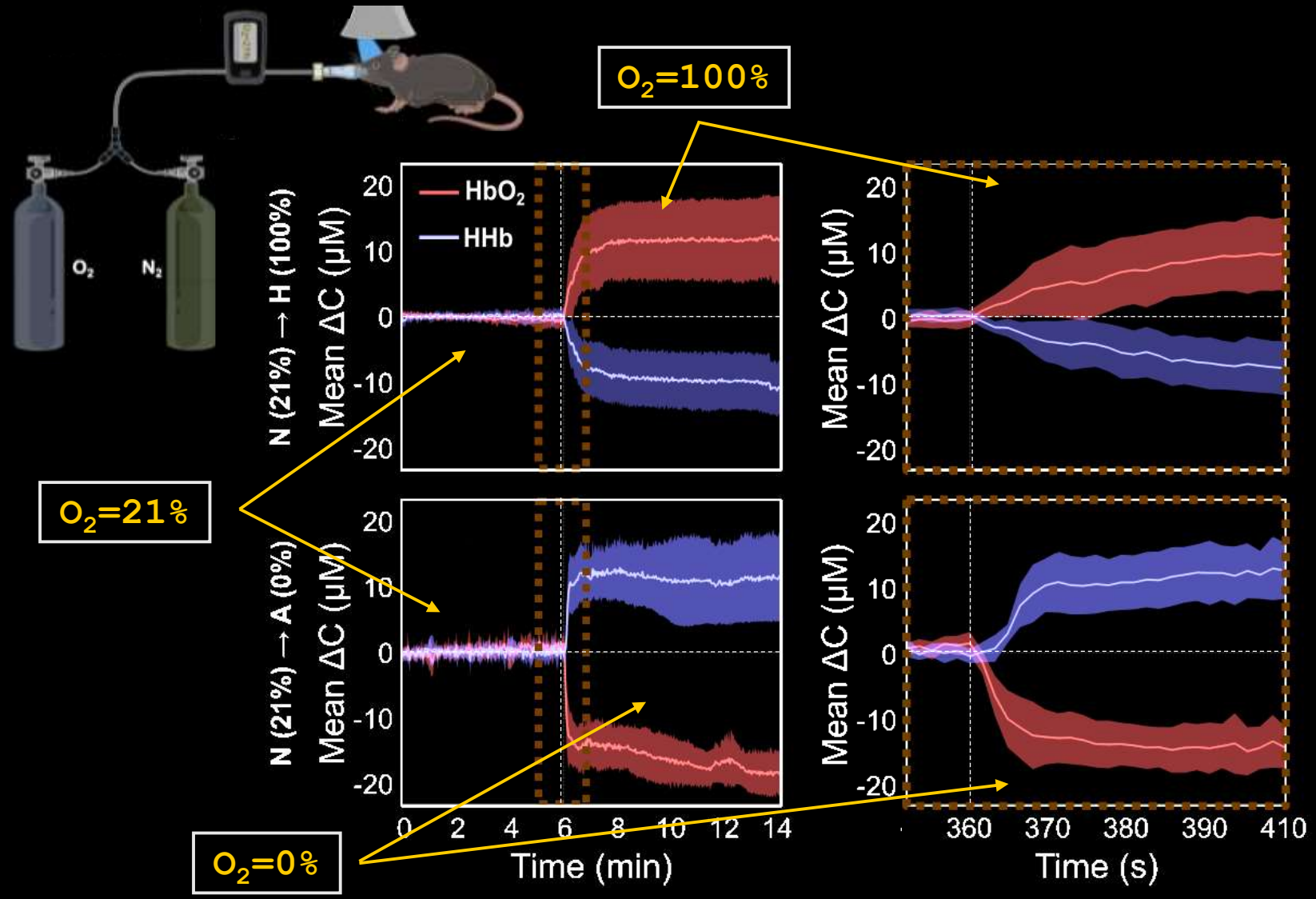

- **Rapid** hemodynamic changes following oxygen modulation
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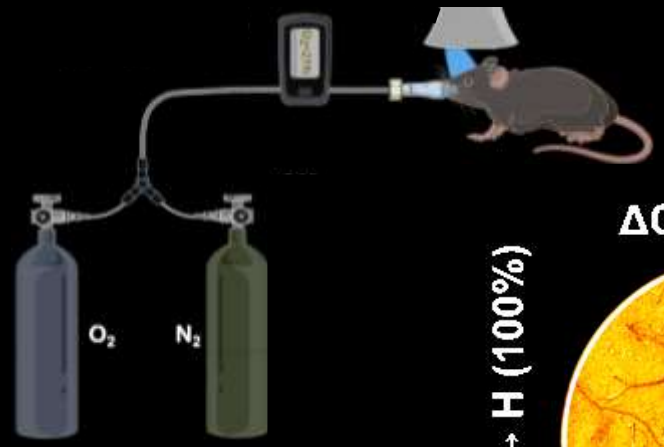
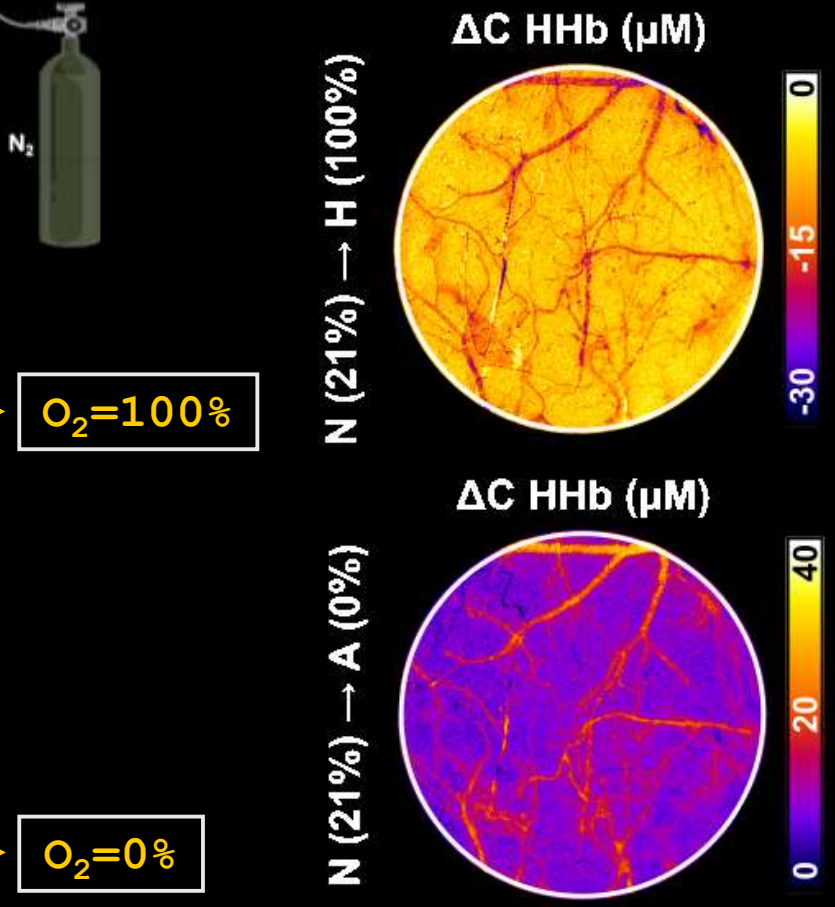
$$Hb(aq) + 4O_2(aq) \rightleftharpoons Hb(O_2)_4(aq)$$

Multispectral imaging enabled **continuous** monitoring of oxygenation transitions, with HbO_2 and HHb changes quantified relative to a normoxic baseline



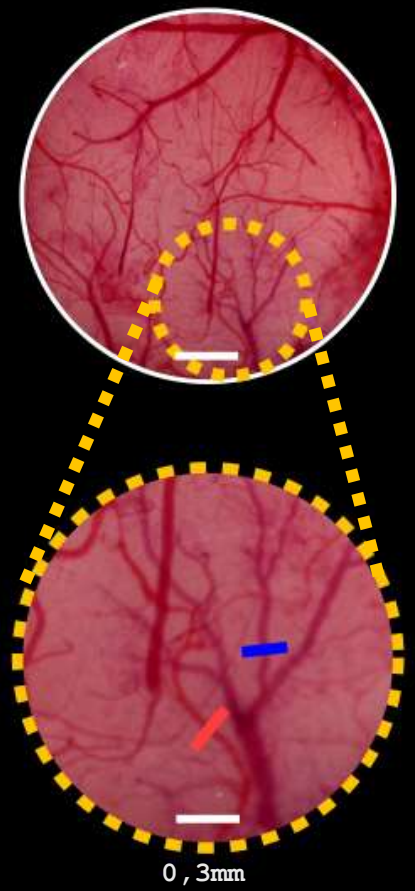
O_2=21% → O_2=100%

O_2=21% → O_2=0%

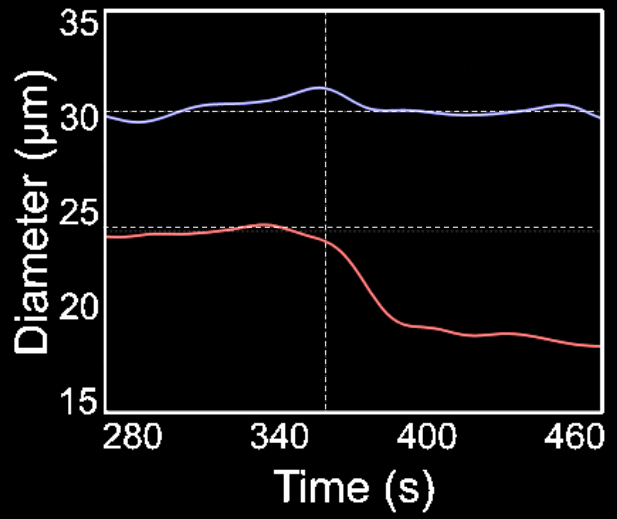


- Distinct **spatial patterns** of HbO_2 and HHb were observed during oxygenation transitions
- Clear differences in concentration variation between **vascular** structures and **surrounding** tissue

- **Arteries** show strong oxygen-dependent diameter changes (**dilation** in anoxia, **constriction** in hyperoxia), while veins remain largely stable
- This reflects active **arterial regulation** of cerebral blood flow versus a more passive venous response



Artery
Vein



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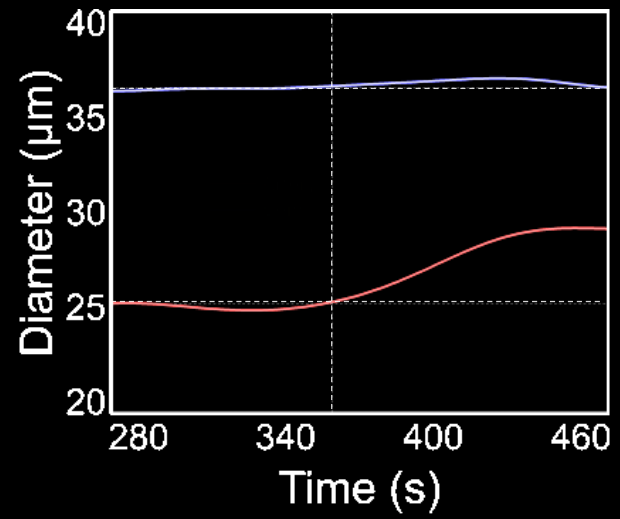

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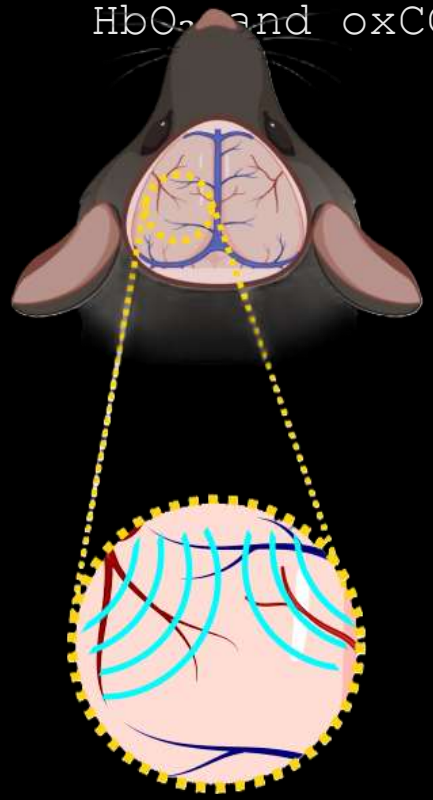
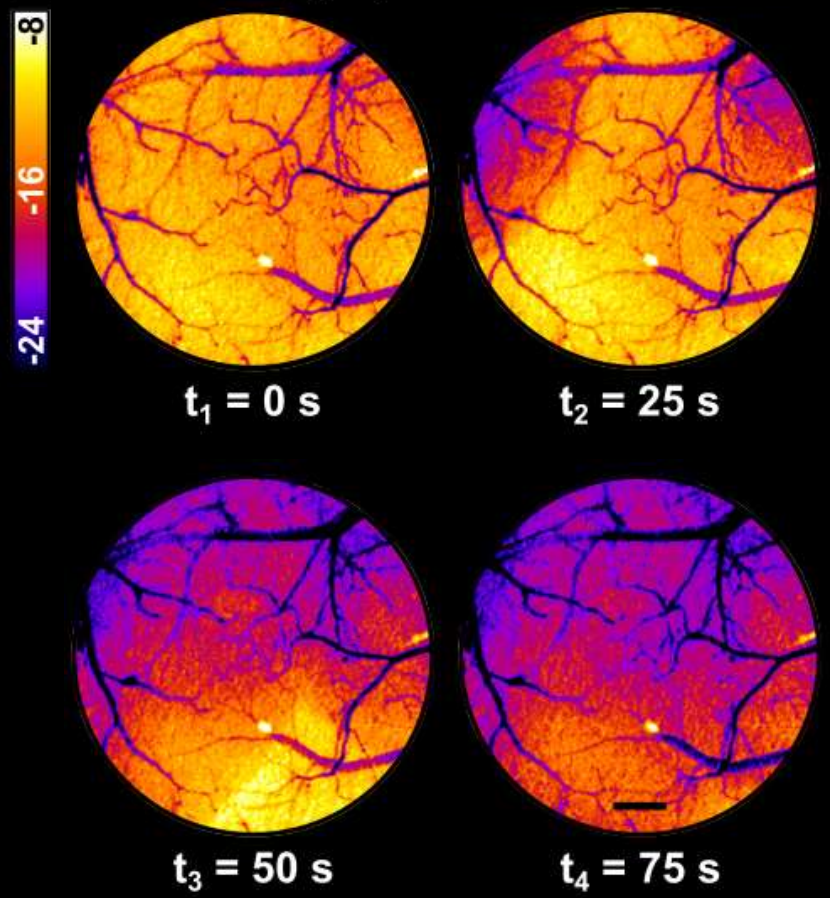
- **Prolonged anoxia** induces propagating **cortical depolarization waves,** indicating severe metabolic failure
- These events spread across the cortex as spatially coherent wavef ased HbO- and oxCCO

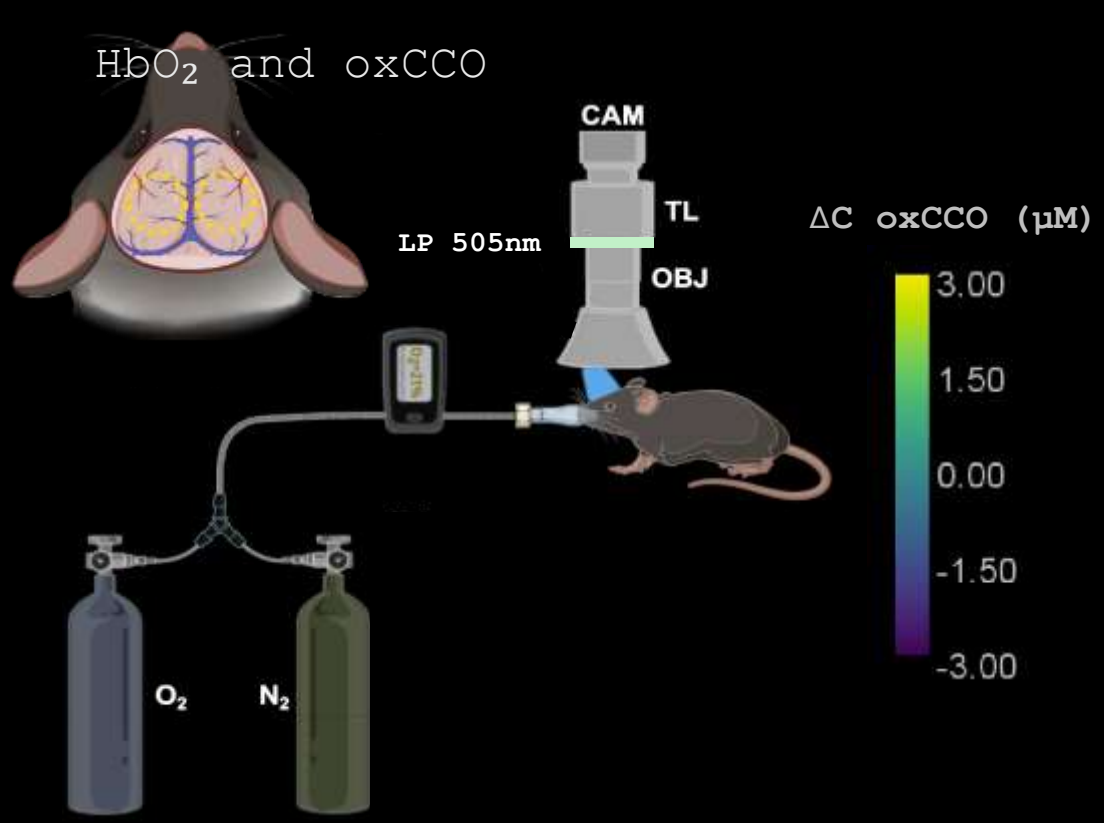


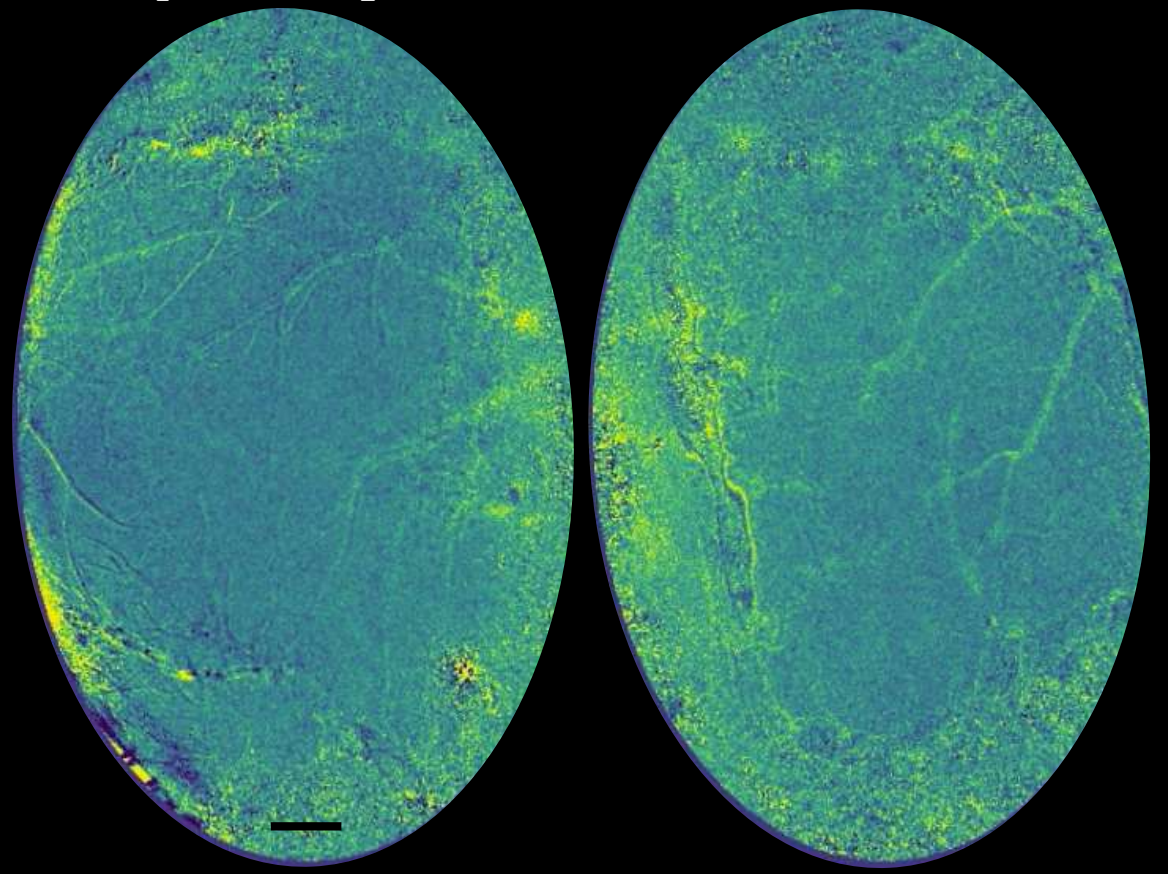
Artery
Vein
Depolarization wave

Δt=2s

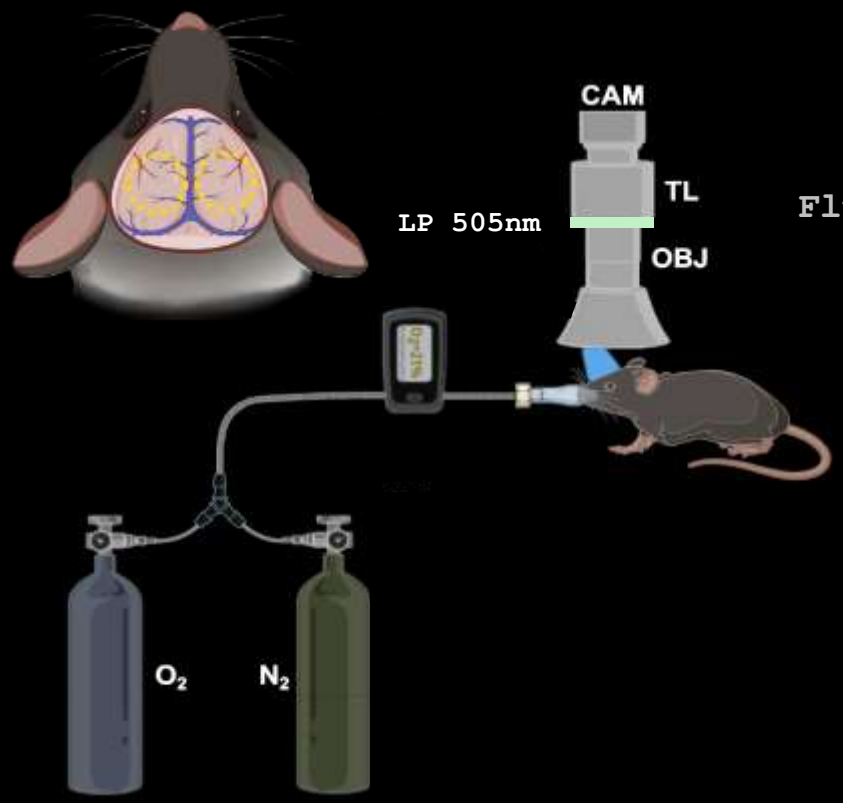


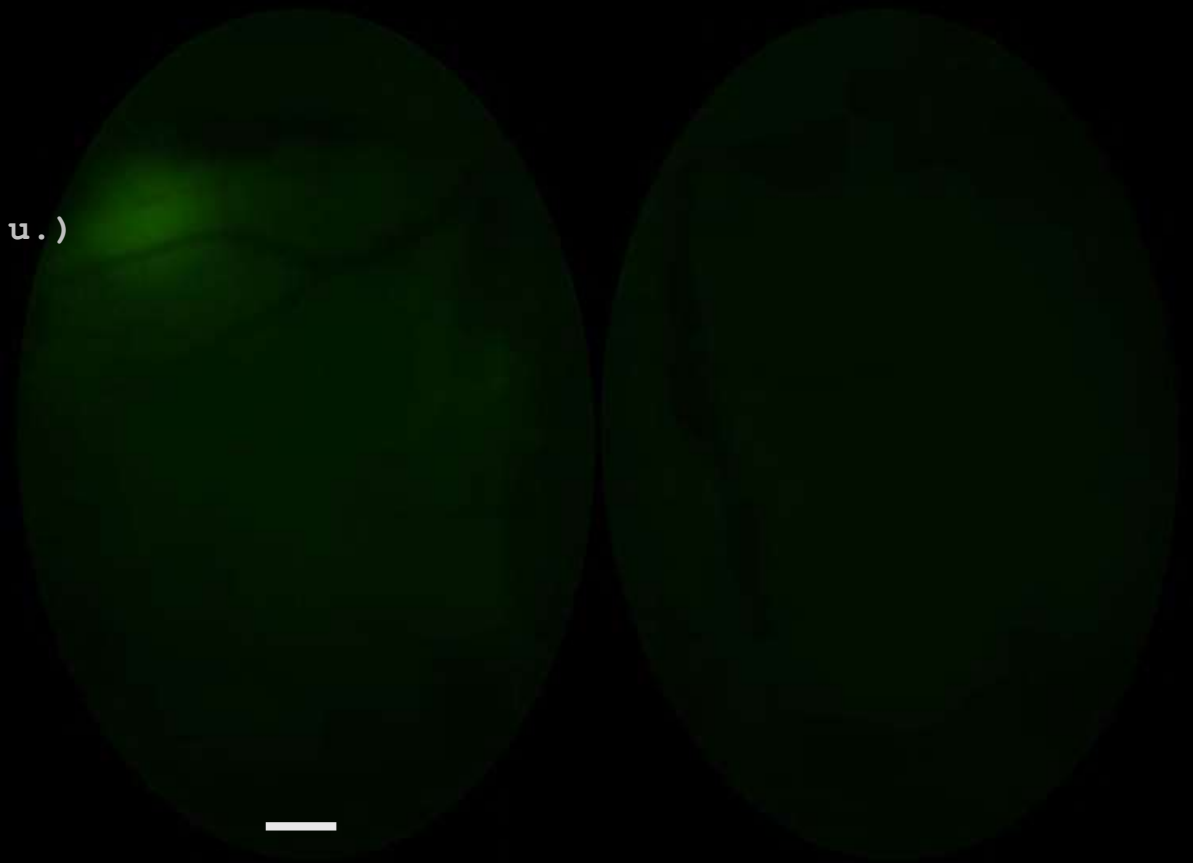
- **Prolonged anoxia** induces propagating **cortical depolarization waves,** indicating severe metabolic failure
- These events spread across the cortex as spatially coherent wavefronts of decreased HbO_2 and oxCCO





Propagating depolarization waves were independently visualized using **GCaMP fluorescence imaging**. Observations support hyperspectral findings of large-scale cortical depolarization under anoxia





- HyperProbe provides a **comprehensive**, **real-time** view of brain physiology
- Bridges the gap between **vascular and metabolic imaging**
- Represents a promising tool for **intraoperative** monitoring and neuronavigation
- **Potential** to improve understanding and management of brain tumors, stroke, and neurodegenerative diseases

Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union. Neither the European Union nor the granting authority can be held responsible for them.

HyperProbe

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UNIVERSITÀ DEGLI STUDI FIRENZE

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THANK YOU

Pietro Ricci
pietro.ricci@unifi.it

www.hyperprobe.eu

The HyperProbe

The HP **laboratory version** is a key first step for testing and optimizing the performances for clinical perspectives

sCMOS camera

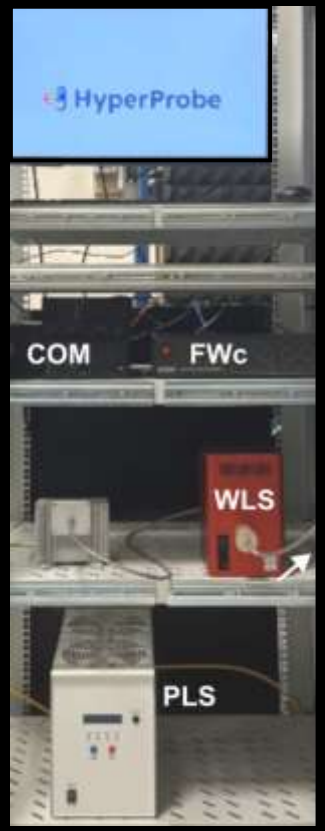
2048 x 2048 pixels
6.5 x 6.5 µm sensor
Peak QE 80%
Max frame rate 40fps

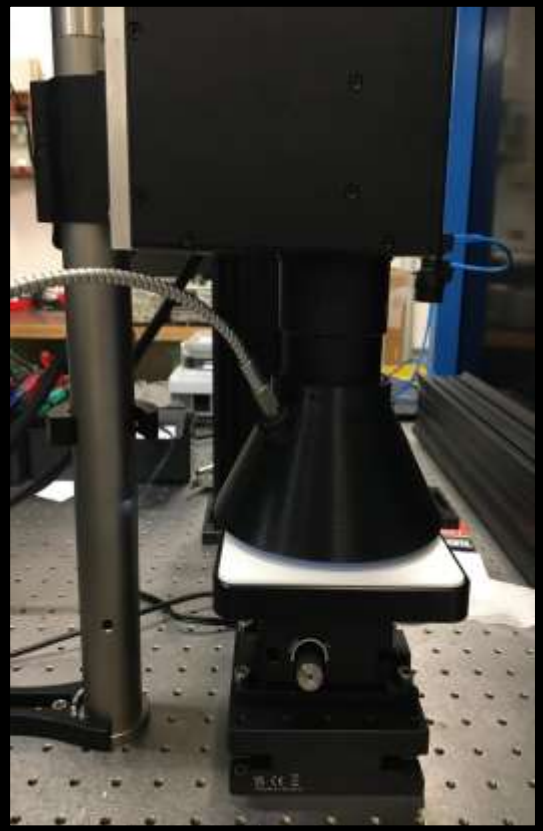
SPECIAL FEATURES XWS-65

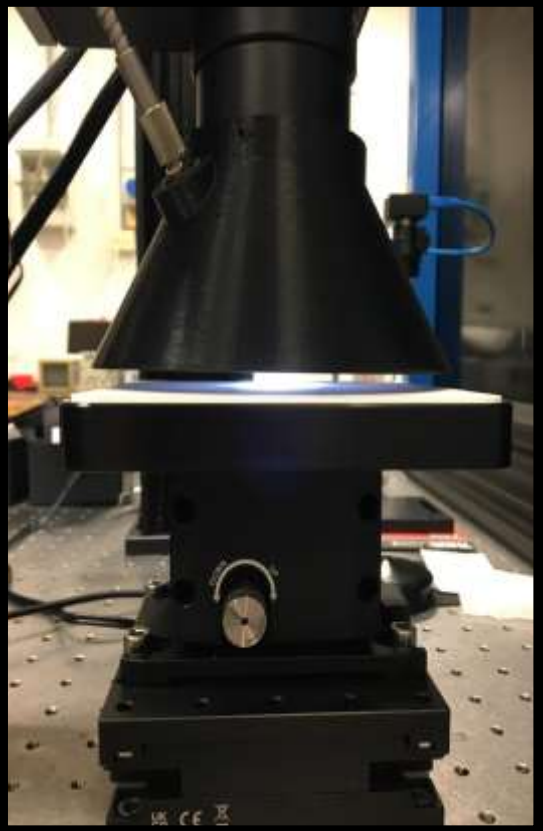
Spectral range 250 - 2500 nm,
Ozone-free (OFR) configuration
Spectral range 190 - 2500nm,
UV configuration
High spectral brightness up to
68 mW/(mm²-sr-nm)
High temporal and spatial stability
STD < 0.15%

Spectrolight, FWS-Poly-CUS-10

Optical Specifications:
Transmission: >75%
Spectral Range: 385-1015 nm
FWHM range: 5-15 nm
Switching time: 10 ms

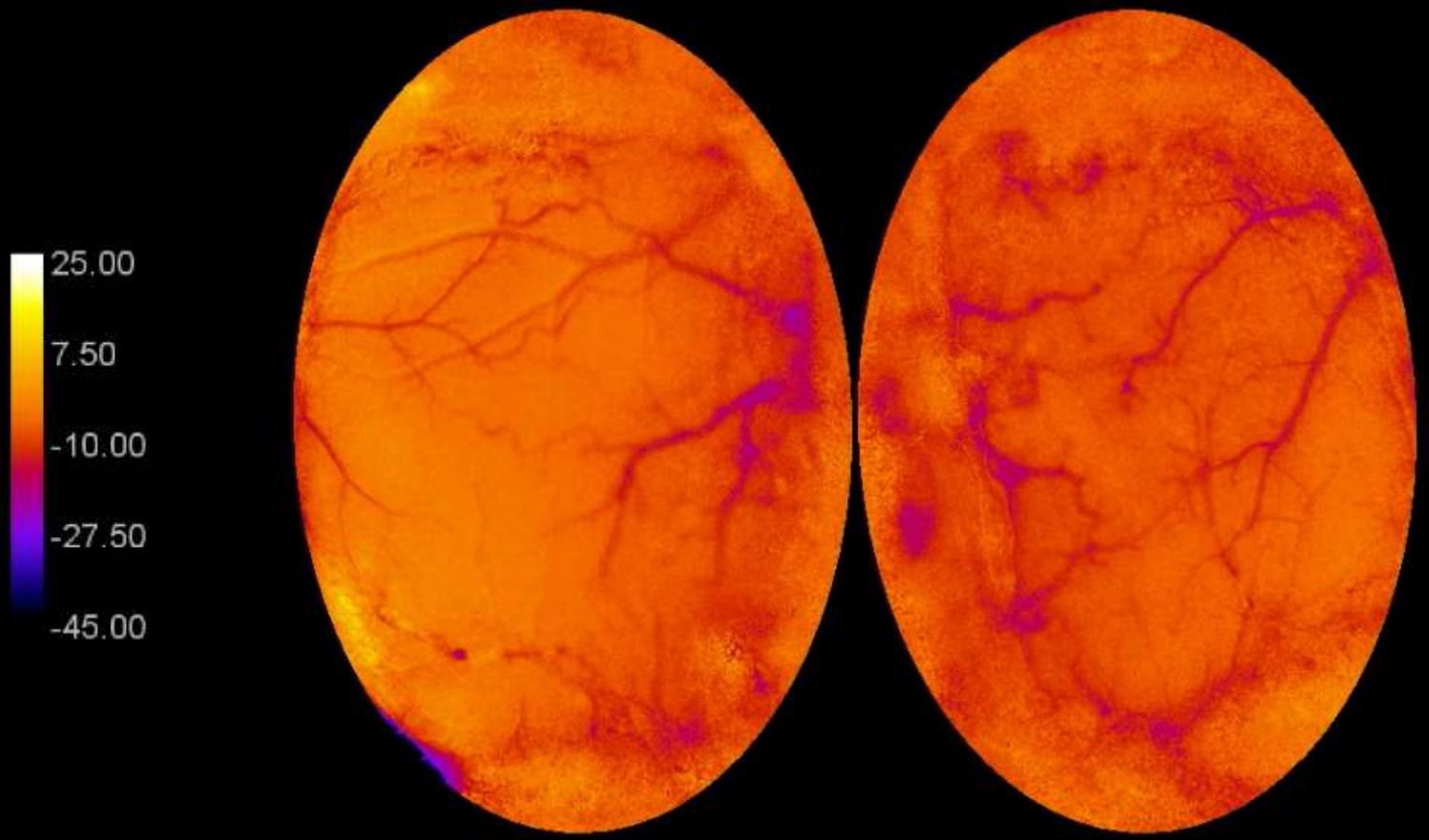






*PLS: plasma light source; WLS: wavelength selector; CAM: camera; FW: filter wheel; OBJ: objective; TL: tube lens; COM: computer; MS: motorized stage

- HyperProbe enables **real-time detection** of large-scale pathological dynamics in vivo
- **Prolonged anoxia** induces propagating **cortical depolarization waves**, indicating severe metabolic failure
- These events s t wavefronts of decreased HbO_2



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