Strategy and Technological Implementations for Concurrent Fluorescence Measurements of Multiple Biological Parameters in Behaving Animals

Inventors at Stanford have developed a novel strategy to perform concurrent fluorescence measurements of multiple biological parameters in freely moving and head-restrained animals. As more genetically encoded fluorescent indicators (GEFI) are being developed to monitor, in living animals, a wide range of biological phenomenon (e.g. transmembrane voltage, ions, neurotransmitters, neuromodulators, opioids, pH...), optical approaches that capture the precise temporal relationship of many physiological processes simultaneously in awake animals become critically necessary. Unlike our invention, none of the existing technologies offers the experimental flexibility to combine multiple fluorescence sensors and monitor those signals.

Two of the most common optical tools in neuroscience to study in vivo brain tissue dynamics during ecologically relevant behavior are fiber photometry and mesoscopic imaging. Fiber photometry is a measurement technique that aggregates fluorescence signal using a fiber optic. While it captures signal at the tip of the fiber, mesoscope imaging will instead capture the spatiotemporal dynamics of the fluorescence signal in awake, head-fixed animals. Recent studies have used both techniques to optically sense voltage, calcium, or neuromodulator dynamics in a variety mouse behavior.

However, none of the state-of-the art fiber photometry devices or 1-photon epifluorescence mesoscope systems are capable of imaging more than 1 biological

parameter at once while precisely referencing biological noises. Indeed, until now, there has not been a suitable method to account for various biological artifacts while imaging 2 spectrally orthogonal fluorescent proteins that capture two independent biological parameters. Therefore, while multiplexing fluorescent signals gain more appeal in the field, molecular strategy and technological implementations to achieve this with high sensitivity and low cost is needed.

Here, we introduce a new molecular strategy and offer two technological implementations for fiber photometry and mesoscope imaging. While the former relies on a lock-in amplification technique, the latter relies on precise control over the illumination activation and imaging frame acquisition. Our simple molecular solution can be used with any fiber photometry systems (used in freely behaving animals) or 1-photon epifluorescence wide-field imaging techniques (used in head-fixed animals) to probe the dynamics of multiple molecular phenomena concurrently in awake animals. This strategy is universally applicable to any fluorescent reporters, from genetically encoded fluorescent proteins to synthetic dyes and nanoparticles. This strategy will reduce by 50% the cost of instrumentations used for fluorescence multiplexing of dynamic processes.

Stage of Development
Research – in vivo – working prototype and proof-of-concept in mice

Related Technology
Stanford Docket S22-228: [Apparatus for Sensitive Fluorescence Optical Measurement of Biological Parameters in Freely Behaving Animals](#)

Applications

- Neuroscience research
- Medical Diagnostics and Treatment Monitoring
- Pharmaceutical Development, Drug discovery platform
- Analytic chemistry

Advantages

- A cost-effective method to multiplex fluorescence signals.

- Simultaneous dual biological parameters with identical performance, in freely and head-restrained behaving mouse.
- Safeguarding against instrumental and/or biological artefacts.
- Universally applicable to any fluorescent reporters, from genetically encoded fluorescent proteins to synthetic dyes and nanoparticles.

Publications

- Simon Haziza, et al. Imaging high-frequency voltage dynamics in multiple neuron classes of behaving mammals. *Cell*, July 16, 2025 (Open access).
- Simon Haziza, et al. PSTR241.29 / WW72 - Optical sensing of high-frequency voltage dynamics in multiple neuron classes of behaving mammals. Poster Presentation, Session PSTR241 - Optical Methodology - Development, Neuroscience 2023.

Patents

- Published Application: 20250064309

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