Hyperspectral fiber photometry for multiplexed neural signal imaging

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Introduction

Motivation. The brain is made up of many cell types that communicate through the release of a variety of neurotransmitters to control brain function and behavior. Genetically encoded fluorescent indicators are widely used to measure neurotransmitter release on specific locations and cell types to dissect neural circuits.

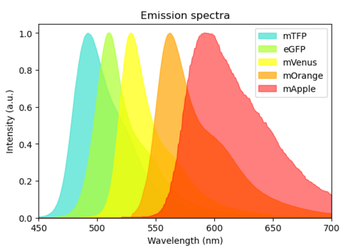
Challenge. Due to technical limitations, prior work has largely been limited to studying at most two signals at once in each brain region.

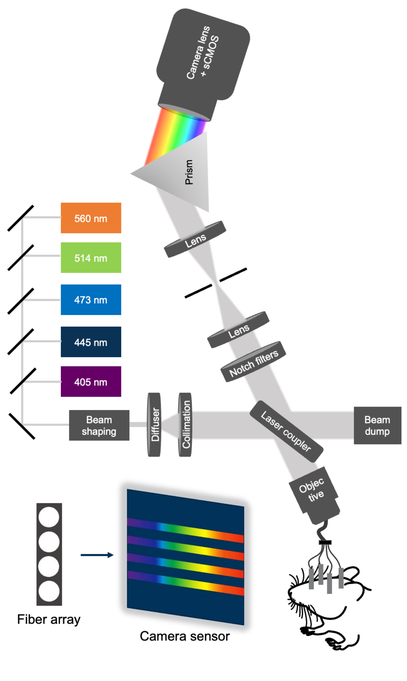
Solution. To enable multiplexed fluorescent measurements across many brain areas in vivo, we developed a hyperspectral fiber photometry system. The system records spectrally resolved emission at five excitation wavelengths. Hyperspectral measurements are unmixed to obtain individual sources of signal using a custom constrained non-negative matrix factorization algorithm.

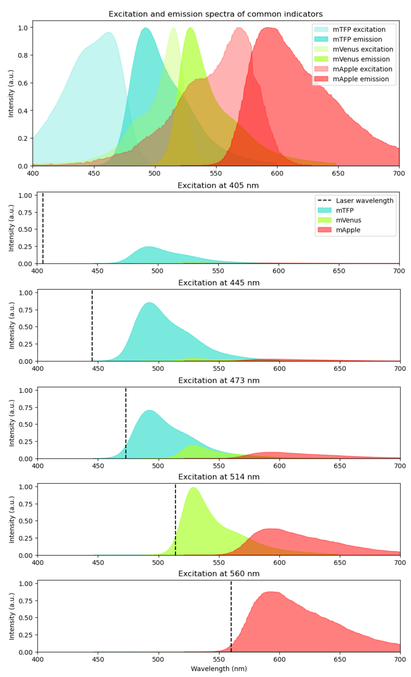
Results. We have performed simultaneous measurements of dopamine, acetylcholine, and calcium in the ventral striatum of mice performing a reward-based decision-making task. Preliminary analysis reveals distinct dynamics of each of these signals modulated by behavioral states and outcomes.

Hyperspectral fiber photometry design

Analyte	Sensor	Color
Dopamine	GRAB-DA, dLight	Green, Red
Serotonin	GRAB-5HT, iSeroSnFR	Green, Red
Acetylcholine	GRAB-ACh, iAChSnFR	Green, Red, Yellow
Norepinephrine	GRAB-NE, nLight	Green
Glutamate	iGluSnFR	Green, Yellow
GABA	iGABASnFR	Green
Voltage	ASAP3, Voltron	Green, Yellow, Red
Calcium	CaMP, GECO	Teal, Green, Yellow, Orange, Red

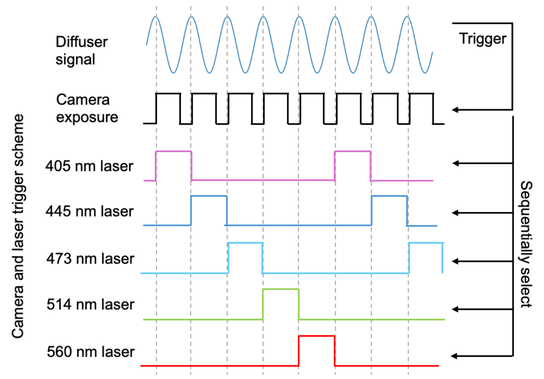




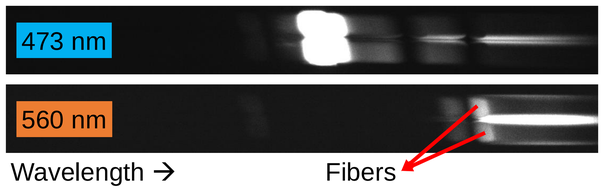


Data acquisition and preprocessing methods for efficient spectral unmixing

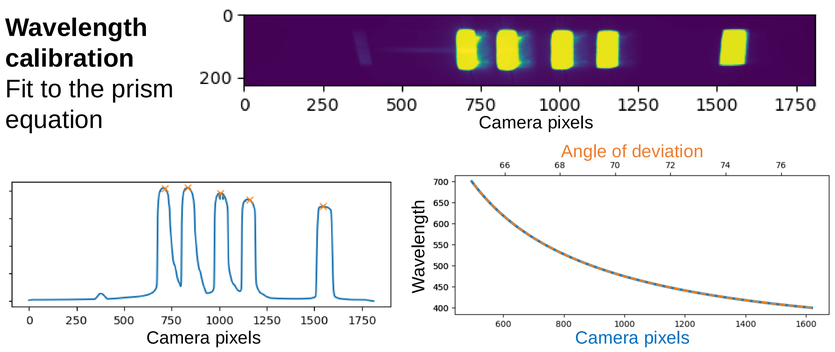
Data acquisition trigger scheme
Spinning diffuser sends trigger signals to camera and lasers



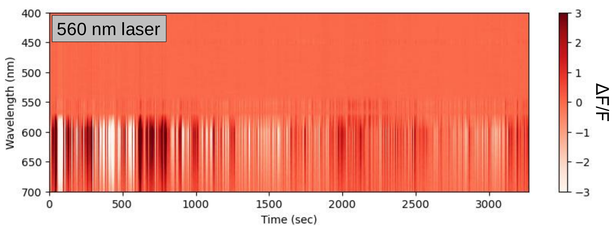
Raw images (intensity adjusted)



Wavelength calibration
Fit to the prism equation



Output
Intensity at wavelength across time.
(One laser shown as example)

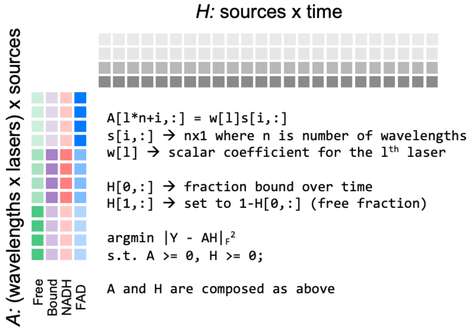


Spectral unmixing using non-negative matrix factorization

Non-negative matrix factorization learns a factorization of the data matrix Y in terms of low-rank, non-negative matrices A and H.

Sources modeled are the excitation lasers, indicators, as well as autofluorescence (e.g. from NADH and FAD).

Objective function L is minimized through projected gradient descent.

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A[l*n+i,:] = w[l]s[i,:]
s[i,:] → nx1 where n is number of wavelengths
w[l] → scalar coefficient for the l^th laser

H[0,:] → fraction bound over time
H[1,:] → set to 1-H[0,:] (free fraction)

argmin |Y - AH|_F^2
s.t. A >= 0, H >= 0;

A and H are composed as above
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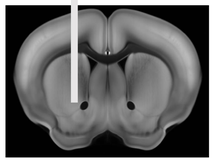
Simultaneous dopamine, acetylcholine, and calcium imaging during behavior

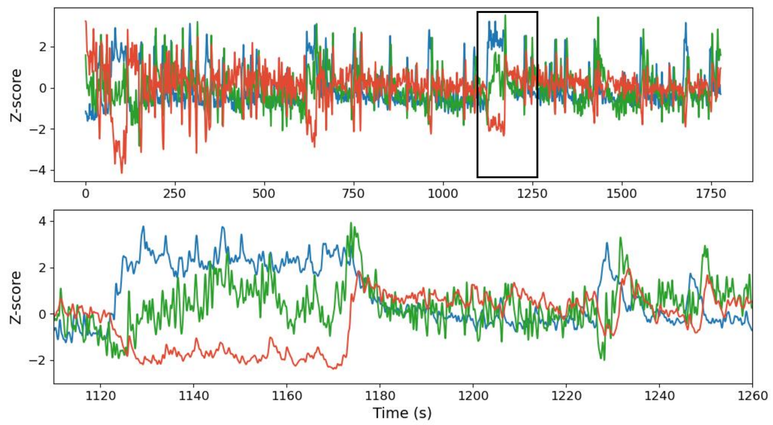
Nucleus accumbens



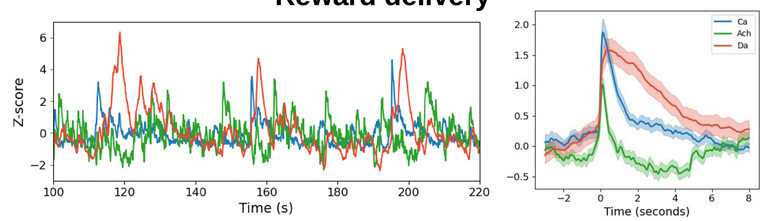
AP: 1.2; ML: -1.3; DV: 4.0

Analyte	Indicator	Peak emission	Expression target
Calcium	TGECO1	492 nm	Neurons
Acetylcholine	iAChSnFR	528 nm	Astrocytes
Dopamine	GRAB-rDA3m	592 nm	Neurons

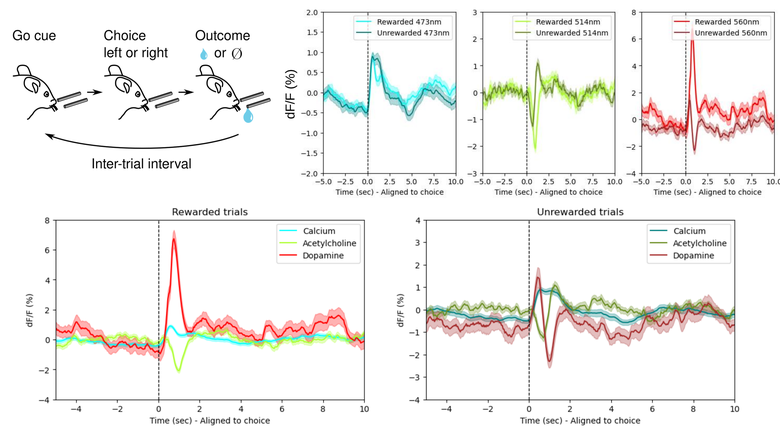
Spontaneous activity



Reward delivery



Dynamic foraging task



References

- Sabatini, B. L., & Tian, L. (2020). Imaging neurotransmitter and neuromodulator dynamics in vivo with genetically encoded indicators. *Neuron*, *108*(1), 17-32.
- Meng, C., Zhou, J., Papaneri, A., Peddada, T., Xu, K., & Cui, G. (2018). Spectrally resolved fiber photometry for multi-component analysis of brain circuits. *Neuron*, *98*(4), 707-717.
- Lee, D., & Seung, H. S. (2000). Algorithms for non- negative matrix factorization. *Advances in neural information processing systems*, *13*.
- Bari, B. A., Grossman, C. D., Lubin, E. E., Rajagopalan, A. E., Cressy, J. I., & Cohen, J. Y. (2019). Stable representations of decision variables for flexible behavior. *Neuron*, *103*(5), 922-933.

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