

Adapting a Two-Photon Scanning Microscope for Simultaneous Single-Photon Imaging of an Infrared Dopamine Sensor

Matthew Tarchick,^{1*} Franklin Caval-Holme,^{1*}  Ben Smith,²  Petra Mocellin,¹ Markita Landry,^{1,3,4}  Natsumi Komatsu,^{4,5**} and  Marla B. Feller^{1**}

¹Department of Neuroscience and Helen Wills Neuroscience Institute, University of California, Berkeley, Berkeley, California 94720, ²School of Optometry, University of California, Berkeley, Berkeley, California 94720, ³California Institute for Quantitative Biosciences, University of California, Berkeley, Berkeley, California 94720, ⁴Department of Chemical & Biomolecular Engineering, University of California, Berkeley, Berkeley, California 94720, and ⁵Department of Bioengineering, University of Illinois Urbana-Champaign, Urbana, Illinois 6180

Abstract

We describe a novel method for adapting a two-photon scanning microscope to enable simultaneous detection of two-photon-generated visible fluorescence and single-photon-generated near-infrared (nIR) fluorescence. In this configuration, nIR fluorescence is routed through a single-mode optical fiber before detection by a photomultiplier tube. This fiber coupling offers two advantages: first, the optical fiber functions as a pinhole aperture, allowing for improved optical sectioning of the nIR signal; second, it minimizes nIR background fluorescence. To validate the effectiveness of this design, we conducted two sets of experiments in male and female C57B/6J mice. First, we compare two fluorescence indicators of the neurotransmitter dopamine: the genetically encoded indicator GRAB_{DA} and single-walled carbon nanotube-based optical nanosensors (nIRCats). Although nIRCats exhibit lower affinity for dopamine than GRAB_{DA}, this property allows for identification of high concentration release sites in the striatum. Second, we simultaneously imaged depolarization-induced calcium changes and dopamine release in the retina. Together, these results demonstrate the utility of integrating confocal nIR detection into a two-photon platform for simultaneous functional imaging across complementary spectral channels.

Significance Statement

Dual-color, real-time imaging is a powerful technique in biomedical imaging, including neuroscience. Here, we present a widely applicable modification to a standard two-photon scanning microscope that adds a near-infrared detection capability, a wavelength range that minimizes photon scattering and autofluorescence from biological samples. Using this microscope, we demonstrate the first direct comparison of two dopamine sensors: the genetically encoded sensor GRAB_{DA3m} detected in the visible channel and carbon nanotube-based sensors detected in the near-infrared channel. We further demonstrate simultaneous imaging of calcium activity and dopamine signaling in the developing retina. While we focused on dopamine sensors in this study, this platform is broadly applicable to a wide range of fluorophores and can be implemented on existing two-photon microscopes.

Introduction

The neuromodulator dopamine plays a critical role in multiple brain functions (Speranza et al., 2021). Understanding the mechanisms by which dopamine impacts brain activity requires measuring spatial and temporal dynamics of extracellular dopamine

Received Jan. 8, 2026; revised March 23, 2026; accepted April 10, 2026.

The authors declare no competing financial interests.

Author contributions: F.C.-H., B.S., M.L., N.K., and M.B.F. designed research; M.T., F.C.-H., B.S., P.M., and N.K. performed research; F.C.-H., B.S., and N.K. analyzed data; F.C.-H., B.S., M.L., N.K., and M.B.F. wrote the paper.

This work was supported by the National Institutes of Health (NIH) R21 EY029405 (M.B.F., M.L.), NIH EY013528 (M.B.F.), NIH EY019498 (M.B.F.), NIH F31EY028022 (F.C.-H.), K99EY037374 (F.C.-H.), and NIH P30EY003176 (B.S.). We acknowledge support of a Burroughs Wellcome Fund Career Award at the Scientific Interface (M.L. and N.K.), a Dreyfus Foundation Award (M.L.), the Philomathia Foundation (M.L.), an NSF CAREER Award 2046159 (M.L.), McKnight Foundation Award (M.L.), a Simons Foundation Award (M.L.), a Moore Foundation Award (M.L.), a Heising-Simons Fellowship (M.L.), a Brain

Continued on next page.

concentrations (Sippy and Tritsch, 2023). For example, diverse firing patterns in the ventral midbrain modulate distinct brain functions: tonic firing in substantia nigra dopaminergic neurons produces sustained elevation of dopamine in the dorsal striatum that promotes a permissive environment for movement (da Silva et al., 2018), while burst firing in the ventral tegmental area generates transient elevations of dopamine associated with reward prediction errors (Bayer and Glimcher, 2005). In the retina, dopamine similarly acts as a critical neuromodulator, regulating short-term light adaptation, modulating receptive field properties, and contributing to circadian changes in retinal function (Witkovsky, 2004; Jackson et al., 2012; Roy and Field, 2019).

To characterize the dynamics of dopamine release, the field has turned to optical sensors. One popular class of these sensors includes genetically encoded sensors in which green fluorescent proteins are integrated with dopamine receptors (dLight and GPCR activation-based dopamine sensors GRAB_{DA}; Patriarchi et al., 2018; Sun et al., 2018). These sensors have proven to be powerful detectors of dopamine release but require viral transduction of the sensors and therefore are limited to use in genetically tractable organisms.

Another tool for detecting dopamine optically is synthetic near-infrared (nIR) catecholamine nanosensors (nIRCat) (Beyene et al., 2019; Del Bonis-O'Donnell et al., 2021). nIRCat nanosensors are synthesized from single-walled carbon nanotubes, which fluoresce in the near-infrared, a window that prevents scattering in tissue. They are functionalized with (GT)₆ DNA sequences to have specificity for dopamine and norepinephrine over other neurotransmitters. nIRCat sensors have a lower affinity for dopamine than the genetically encoded sensors (Beyene et al., 2019), but they allow for the detection of hotspots of dopamine release, such as in slices of the dorsal striatum. In addition, nIRCats have been incorporated into films, allowing for the characterization of dopamine release along isolated dopaminergic axons (Bulumulla et al., 2022; Elizarova et al., 2022).

Furthermore, nIRCat's near-infrared fluorescence is spectrally well separated from visible fluorophores, providing an opportunity to study dopamine release simultaneously with calcium dynamics or other neurotransmitter activities. Dual imaging of nIRCat and visible wavelength fluorophores has been demonstrated (Bulumulla et al., 2022; Elizarova et al., 2022); however, visible wavelength fluorophores were used to label neurons in those studies, rather than to monitor real-time activity.

Here we introduce a dual-modal scanning microscope that integrates two-photon excitation of a visible fluorophore with single-photon excitation of nIR nanosensors. Optical channels are spectrally resolved and independently coupled to maximize signal fidelity. We validated our design by measuring real-time dopamine dynamics in the striatum with simultaneous imaging of nIRCats and GRAB_{DA3m}. We further demonstrate real-time imaging of depolarization-induced calcium transients and simultaneous dopamine release in the retina. While we focus on monitoring sensors of Ca²⁺ and neurotransmitter release as a proof of concept in this study, this framework generalizes to any visible fluorophores and supports flexible expansion to other analytes or modalities.

Materials and Methods

Experimental model. All experiments were performed in male and female C57B/6J mice (Jackson Laboratory). The animals were maintained on a 12 h light cycle (lights on at 07:00). All animal procedures were performed in accordance with the University of California Berkeley Institutional Animal Care committee's regulations and Use Committees and conformed to the National Institutes of Health's Guide for the care and use of laboratory animals, the Public Health Service Policy, and the Society for Neuroscience Policy on the Use of Animals in Neuroscience Research.

Stereotactic viral injection. The injections were performed in 6–8 week-old mice under general ketamine–dexmedetomidine anesthesia using a stereotaxic instrument (Kopf Instruments, Model 1900) as previously described (Lammel et al., 2008). For the GRAB_{DA} experiment, 400 nl of AAV2/9-hSyn-GRAB_{DA3m}-WPRES-hGH polyA (BrainVTA, PT-4720) was injected bilaterally in the dorsal striatum. The coordinates used to target the dorsal striatum were AP: +1; ML: ±1.5; DV: −3.0 relative to bregma and based on the Allen Mouse Brain Atlas. After all injections, the needle was left in place for at least 5 min before withdrawal. The animals were kept on a heating pad until recovered from anesthesia. Experiments were performed 3–4 weeks after stereotactic injection.

Foundation Award (M.L.), a Polymaths award from Schmidt Sciences, LLC (M.L.), Schmidt Science Fellows, in partnership with the Rhodes Trust (N.K.), and EMBO Long-Term Postdoctoral Fellowship (P.M.). M.L. is a Chan Zuckerberg Biohub investigator.

*M.T. and F.C.-H. contributed equally to this work.

**N.K. and M.B.F. contributed equally to this work.

Correspondence should be addressed to Natsumi Komatsu at komatsu@illinois.edu or Marla B. Feller at mfeller@berkeley.edu.

Copyright © 2026 Tarchick et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license, which permits unrestricted use, distribution and reproduction in any medium provided that the original work is properly attributed.

Acute brain slice preparation. Three to four weeks after viral injection, mice (10–12 weeks old) were deeply anaesthetized with pentobarbital (200 mg/kg, i.p.; Vortech). After intracardial perfusion with ice-cold artificial cerebrospinal fluid [aCSF; containing the following (in mM): 119 NaCl, 26.2 NaHCO₃, 2.5 KCl, 1 NaH₂PO₄, 3.5 MgCl₂, 10 glucose, and 0 CaCl₂], the brain was rapidly extracted and sliced with a Leica VT1200 S Vibratome. Before use, slices were incubated at 37°C for 60 min in oxygen-saturated aCSF (in mM: 119 NaCl, 26.2 NaHCO₃, 2.5 KCl, 1 NaH₂PO₄, 1.3 MgCl₂, 10 glucose, and 2 CaCl₂) and then transferred to room temperature for 30 min before nIRCat labeling and imaging experiments.

Retina preparation. Postnatal day (P) 4–8 mice were deeply anesthetized with isoflurane inhalation and killed by decapitation. Eyes were immediately enucleated, and retinas were dissected in oxygenated (95% O₂, 5% CO₂) aCSF (in mM: 119 NaCl, 2.5 KCl, 1.3 MgCl₂, 1 K₂HPO₄, 26.2 NaHCO₃, 11 D-glucose, and 2.5 CaCl₂) at room temperature under white light. In some experiments, 0.1 mM sulforhodamine 101 (SR101, Invitrogen) was added for visualization of vasculature. Each isolated retina was cut into two pieces. Each piece of retina was mounted over a 1–2 mm² hole in nitrocellulose filter paper (Millipore) with the photoreceptor layer side down, dark-adapted for 1 h, and transferred to the recording chamber of a two-photon microscope for imaging. The whole-mount retinas were continuously perfused (3 ml/min) with oxygenated aCSF warmed to 32–34°C by a regulated inline heater (TC-344B, Warner Instruments) for the duration of the experiment. Additional retina pieces were kept in the dark at room temperature in aCSF bubbled with 95% O₂, 5% CO₂ until use (maximum 8 h).

nIRCat synthesis. nIRCATs were prepared following a previously developed protocol (Beyene et al., 2019). Briefly, HiPco SWCNT slurry (NanoIntegris) and ssDNA (Integrated DNA Technologies) were mixed with a final NaCl concentration of 10 mM. The mass ratio of DNA to SWCNT was 2:1. The DNA sequence was GTGTGTGTGTGT. The mixture was probe-tip sonicated (Cole-Parmer Ultrasonic Processor, 3 mm tip) in ice-cold water for 30 min at 50% amplitude. The resulting suspensions were centrifuged at 21,000 × *g* for 4 h at 4°C and the supernatant was collected. Nanosensors were diluted to 200 mg/L in 10 mM NaCl and stored at 4°C.

nIRCat labeling (brain slices). aCSF (in mM: 119 NaCl, 26.2 NaHCO₃, 2.5 KCl, 1 NaH₂PO₄, 1.3 MgCl₂, 10 glucose, 2 CaCl₂, all purchased from Sigma-Aldrich) was prepared and bubbled with carbogen gas (oxygen/carbon dioxide 95% O₂, 5% CO₂, Praxair). To label the slice with nIRCat, brain slices containing striatum were transferred to an incubation chamber filled with carbogen-bubbled aCSF at room temperature. nIRCATs were applied to the surface of brain slices with a pipette to a final concentration of 2 mg/L and incubated for 15 min. Slices were rinsed for 5 s with bubbled aCSF through 3 wells of a 24-well plate to remove unlocalized nanosensors and rested for at least 15 min before imaging.

nIRCat and Cal 520 labeling (retina). Retinas were transferred to the recording chamber of a two-photon microscope. nIRCat sensors (10 mg/ml in aCSF) were combined 1:1 with Cal 520 AM (AAT Bioquest) and bulk-loaded into the retina using a multicell bolus loading technique (Stosiek et al., 2003; Blankenship et al., 2009). Bolus loading was targeted to the retina's inner plexiform layer so as to deposit the sensor near processes of dopaminergic amacrine cells. Retinas were allowed to equilibrate for 1 h before imaging.

Imaging. Samples were imaged on a modified two-photon microscope, where a near-infrared detection arm was added to the descanned laser path, allowing for simultaneous single-photon near-infrared confocal imaging (design files with parts list are provided here: https://github.com/Llamero/Two-Photon_nIR_descanned_confocal_arm). Carbogen-bubbled aCSF was perfused through the microscope chamber. Slices labeled with nIRCATs were placed in the chamber with a tissue harp, and a glass pipet or a bipolar stimulation electrode (MicroProbes for Life Science Stereotrodes Platinum/Iridium Standard Tip) was positioned to the targeted field of view, which was adjusted with a 4× objective followed by a 60× objective (Olympus 1.00 NA LUMPlanFLN). The microscope was controlled by ScanImage Software. Fluorescent images were acquired at a frame rate of 1.47 frames/s in a 130 μm² (256 × 256 pixel) field of view. An ultrafast pulsed laser (tuned to 920–950 nm) provided fluorescence excitation.

To measure depolarization-evoked dopamine release, 1 s of 100 mM potassium solution or 1 ms of 0.1–0.3 mA stimulation was applied, and this stimulation was repeated at least three times with the same field of view, with 5 min between each stimulation. For the pharmacological measurements, 10 μM of quinpirole (Fisher Scientific, 10-611-0) was applied to the chamber through aCSF perfusion, and slices were incubated for 15 min before imaging. Images were acquired in the same field of view as the one before quinpirole application.

Image analysis—striatal GRAB_{DA} and nIRCat imaging. Imaging movie files were processed using a Python script (<https://github.com/NicholasOuassil/NanolmgPro>). Fluorescent modulation $\Delta F/F_0$ was calculated as $\Delta F/F = (F - F_0) / F_0$, where F_0 is the average intensity before the stimulation and F is the dynamic fluorescence intensity from the entire field of view. Next, an 8 × 8 pixel (corresponding to 6 μm by 6 μm) grid mask was applied to the image stack to minimize bias and improve stack processing time. For each grid square, $\Delta F/F_0$ was calculated, and they were identified as a region of interest (ROI) if the $\Delta F/F_0$ after stimulation was 2 standard deviations above the baseline activity. All the parameters reported in this study are averaged over at least three stimulations.

To compare the time course of the fluorescence responses for nIRCats and GRAB_{DA3m}, the $\Delta F/F_0$ data was fitted to the following:

$$\alpha \times \left(1 - \exp\left(-\frac{x}{\tau_{\text{ON}}}\right)\right) \times \left(\exp\left(-\frac{x}{\tau_{\text{OFF}}}\right)\right) + \beta,$$

where α is a scale factor, β is a constant, τ_{ON} is the rise time constant, and τ_{OFF} is the decay time constant. α , β , τ_{ON} , and τ_{OFF} were fitting parameters.

Image analysis—retinal Ca^{2+} and nIRCats imaging. For each field of view, a single ROI was defined and fluorescence data extracted from both spectral channels in FIJI. ROIs encompassed the entire field of view or the region labeled with nIRCats sensor. Wave-triggered and K^+ puff-triggered average responses were computed using a Python script (<https://github.com/FellerLabCodeShare/nIR-catecholamine-sensor>). Fluorescence signals were low-pass filtered at 0.68 Hz using a third-order Butterworth filter. Fluorescent modulation $\Delta F/F_0$ was calculated as $\Delta F/F = (F - F_0)/F_0$, where F_0 is the median intensity during the imaging trial. In some cases, nIRCats fluorescence exhibited an exponential decay during the first 30–60 s of imaging. These decays were removed by fitting and then subtracting a double exponential. Spontaneous retinal waves were identified by Z-scoring the Cal 520 signal and finding peaks that exceeded the mean by >3 standard deviations and were separated by at least 10 s. Wave and puff-triggered averages were computed within a 60 s window centered on the frame prior to the wave peak or K^+ puff. Responses to puffs and waves were measured from the triggered averages by computing the difference between the peak values (a mean within a 3 s window centered on the peak) in 10 s windows before and after the event trigger.

Code accessibility. Python-based image processing and analysis applications are available at <https://github.com/NicholasOuassil/NanolmgPro> and <https://github.com/FellerLabCodeShare/nIR-catecholamine-sensor>. The code is publicly accessible and does not require special permissions; no accession number is applicable. Analyses were performed using Python (version 3.12.12) on macOS-based systems and in cloud-based environments using Google Colaboratory. The code was executed on standard workstation hardware, and required packages and installation instructions are provided in the repository.

Results

Integrating an infrared detection pathway into a scanning two-photon microscope

nIRCats sensors fluoresce in the near-infrared with a broad range of excitation bands (Cambré et al., 2025). Thus far, imaging of these sensors in brain tissue has been based on epifluorescent imaging, using InGaAs cameras that are sensitive to long wavelengths (Yang et al., 2021). Here we present a strategy for integrating an infrared detection pathway into a two-photon (2P) laser scanning microscope (tuned 920–950 nm), which will allow for multiplexed imaging of these sensors with visible wavelength sensors.

We integrated an indium arsenide (InAs) photomultiplier tube (PMT, Hamamatsu H12397A-75), well established for low-noise infrared detection, into a scanning 2P microscope. The InAs nIR PMT was integrated in the descanned path of the microscope, allowing for the simultaneous imaging of the 2P excitation of visible wavelength sensors and the single-photon confocal imaging of the nIRCats sensors (Fig. 1A,B). Specifically, a 1,100 nm short-pass dichroic mirror was inserted into the two-photon excitation path immediately upstream of the scanning mirrors. This dichroic transmits the two-photon excitation beam while reflecting the near-infrared (nIRCats) emission. The reflected nIR fluorescence then passes through a 1,300 nm long-pass filter to reject residual reflected excitation light before being focused into a single-mode optical fiber. The fiber acts as a confocal pinhole, rejecting out-of-focus fluorescence from above and below the focal plane and guiding the nIR signal to the InAs photomultiplier tube for detection.

To assess the z-sectioning obtained by the optical fiber, we imaged a thin film of lead sulfide quantum dots adhered to a glass slide using both a 50- μm -thick optical fiber and a 200- μm -thick optical fiber (Fig. 1C). We estimated the effective axial resolution as measured at the FWHM of the z-intensity profile of the resulting image stack. For thick fibers, this corresponds to 100 μm while for the 50 μm fibers it was 50 μm . This limited confocality allows for integration of IR signal over the full range of nIRCats sensors in the tissue.

Dual real-time imaging of nIRCats and GRAB_{DA} in dorsal striatum

To demonstrate the dual-imaging capability of our microscope, we simultaneously imaged evoked dopamine release in acute brain slices using both nIRCats and GRAB_{DA}. We prepared coronal slices from mice expressing GRAB_{DA3m} in the dorsal striatum (Fig. 2A–C), a region chosen for its dense dopaminergic innervation and lack of norepinephrine inputs (Berridge and Waterhouse, 2003) as nIRCats are also sensitive to norepinephrine (Beyene et al., 2019; Del Bonis-O'Donnell et al., 2021). We then incubated slices with nIRCats for 15 min before imaging. Using both visible and nIR channels, we observed robust fluorescent signals in both sensors (Fig. 2D). We note that the spatial distribution of GRAB_{DA3m}

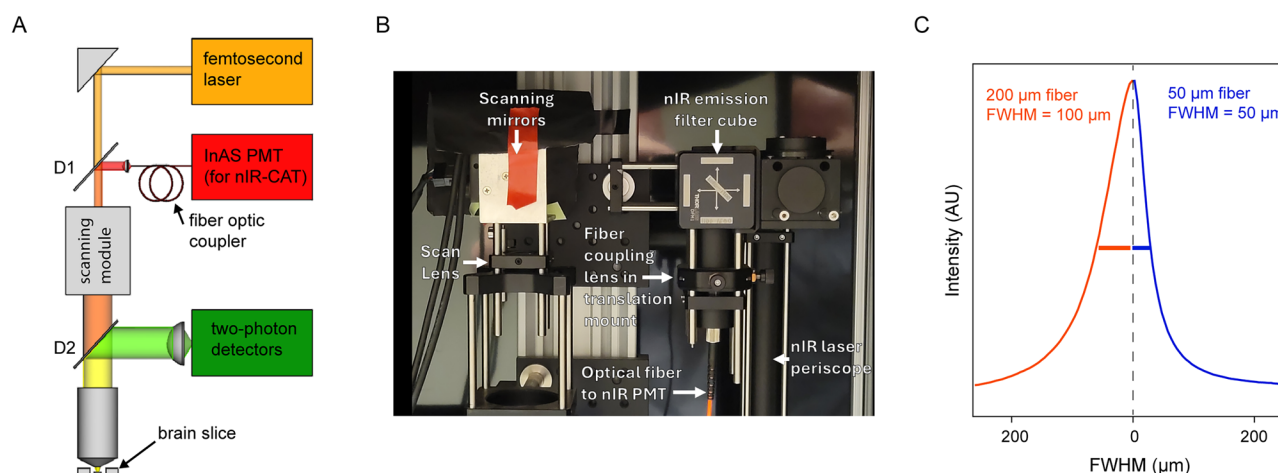


Figure 1. Integrating an infrared detection pathway for imaging of nIRCat fluorescence. **A**, Microscope schematic. A one-photon infrared (IR) detection arm (red) was added to a scanning two-photon microscope. Dichroics: D1: custom dichroic is 680–1,040 short pass, reflecting >1,100 nm. D2: 680 nm long-pass. There is a 1,300 nm low-pass before the InAs PMT. **B**, A photo of the near-infrared descanned detection arm for the two-photon microscope. **C**, Limited z-section provided by the aperture of the fiber optic coupler. Fluorescence profiles of IR fluorescence from lead sulfide quantum dots for a thicker fiber (red, 200 μm) and a thinner fiber (blue, 50 μm). FWHM was 100 μm for the thick fiber and 50 μm for the thin fiber.

and nIRCat did not completely overlap (Fig. 2D), likely because GRAB_{DA3m} is expressed on cell membranes (Sun et al., 2018) whereas nIRCats reside in the brain extracellular space.

We next electrically evoked dopamine release to assess the microscope's dual, real-time imaging performance. Depolarization with bipolar stimulating electrodes evoked increases in fluorescence ($\Delta F/F_0$) in sensors; both GRAB_{DA3m} and nIRCat responded reliably to repeated stimulation (Fig. 2E), confirming their reversibility.

We directly compared the sensor performance of nIRCats and GRAB_{DA3m}: $\Delta F/F_0$ of nIRCats monotonically increased with $\Delta F/F_0$ of GRAB_{DA3m} (Fig. 2F). The $\Delta F/F_0$ of nIRCats was approximately three orders of magnitude smaller than that of GRAB_{DA3m}, consistent with the sensors' different affinities [estimated to be tens of μM for nIRCats (Beyene et al., 2019) and tens of nM for GRAB_{DA3m} (Zhuo et al., 2024)]. In addition to electrical stimulation, both sensors showed robust responses to short applications of potassium (K^+ ; Fig. 2G,H). The nIRCat $\Delta F/F_0$ values exhibited slightly higher variability than GRAB_{DA3m} across brain slices, likely due to changes in loading and the lower signal-to-noise. Note that the $\Delta F/F_0$ magnitude of nIRCats imaged here was one order smaller than prior reports (Beyene et al., 2019; Del Bonis-O'Donnell et al., 2021), likely because of differences in excitation and detection settings and differences in detector quantum efficiency above 1,100 nm.

We further analyzed the time constants of both sensors (Fig. 3). By analyzing integrated $\Delta F/F_0$, averaged over the entire field of view ($74 \mu\text{m} \times 74 \mu\text{m}$; Fig. 3A,B), nIRCat signals exhibit a consistently longer rise time and decay time (Fig. 3C,D). To examine the variability of the response kinetics across the sample, we computed the time constant of the responses within a $6 \times 6 \mu\text{m}$ grid mask. While the modes of both sensors overlap for τ_{ON} and are comparable for τ_{OFF} , nIRCat responses displayed greater heterogeneity (Fig. 3E,F), consistent with the previous study (Beyene et al., 2019). This difference in kinetics likely reflects their volumetric sampling of dopamine throughout the extracellular space, combined with the more diffuse optical sectioning in our one-photon imaging. To our knowledge, this study provides the first direct comparison between GRAB_{DA3m} and nIRCat performance under identical experimental conditions.

nIRCat's lower affinity enables spatial mapping of high dopamine release sites in dorsal striatum

The dorsal striatum is densely innervated by dopaminergic neurons, resulting in a high extracellular dopamine concentration upon depolarization. Although the high affinity of GRAB_{DA3m} is ideal for many applications, including in vivo imaging, it presents a limitation in dopamine-rich regions where the sensor can become saturated, compromising its suitability for spatially resolved analyses. Note, GRAB_{DA} sensors with differing affinities have been introduced to address this issue (Zhuo et al., 2024), but their K_d is still in the nM range. In contrast, the lower affinity of nIRCats allows for identification of localized areas of high dopamine release, including subcellular somatodendritic release as reported earlier (Bulumulla et al., 2022).

To identify putative dopamine release sites, we applied a $6 \times 6 \mu\text{m}$ grid mask to the images from both GRAB_{DA3m} and nIRCat channels and calculated $\Delta F/F_0$ for each grid square (Fig. 4A). Regions of interests (ROIs) were defined as grid squares where peak $\Delta F/F_0$ exceeded two standard deviations above baseline. Using this approach, nIRCat imaging identified ~150 ROIs (out of 361 grid squares) corresponding to "high dopamine spots" (Fig. 4A). In contrast, all 361 ROIs were active in the GRAB_{DA3m} images, consistent with the sensor's high affinity and therefore saturation. Next, to assess the sensitivity of these ROIs to changes in dopamine concentration, we applied quinpirole, a D2 dopamine receptor agonist known to decrease

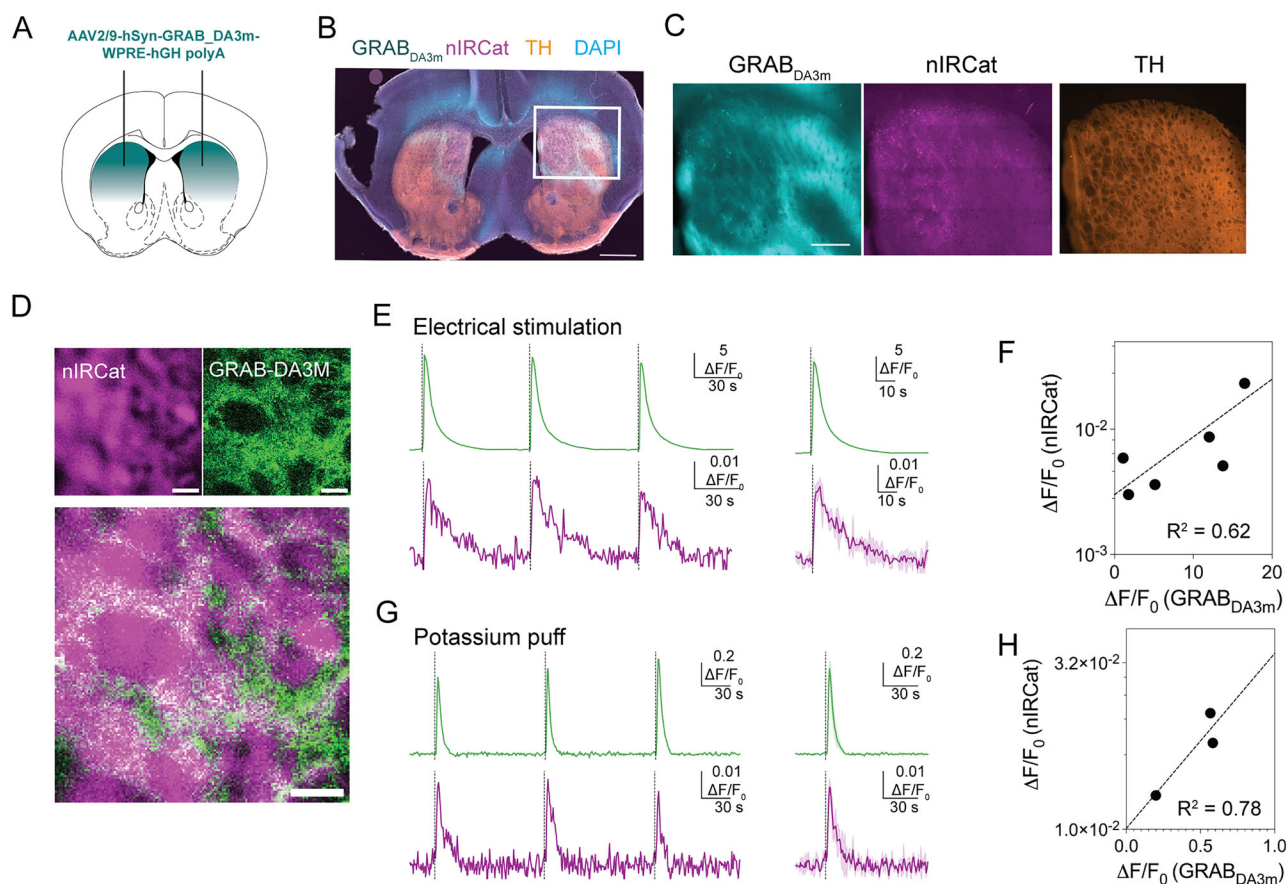


Figure 2. Direct comparison of response amplitudes of nIRCats and GRAB_{DA3m} to evoked dopamine release. **A**, Scheme of the GRAB_{DA3m} injection sites in the dorsal striatum. **B**, Representative striatal slice showing GRAB_{DA3m} viral expression in cyan, putative nIRCats signal in magenta (far red wavelength), and tyrosine hydroxylase (TH) antibody staining in orange. Scale bar, 1 mm. **C**, Same as **B** at a higher magnification. Scale bar, 500 μ m. **D**, Fluorescence images from the nIR channel (nIRCats; left), from the visible channel (GRAB_{DA3m}; right), and their overlay images (bottom). Scale bar denotes 20 μ m. **E**, Representative fractional change ($\Delta F/F_0$) in fluorescence of GRAB_{DA3m} (top, green) and nIRCats (bottom, purple), integrated from a 74 μ m $\times$ 74 μ m field of view, in response to depolarization via 300 μ A electrical stimulation. Individual response (left) and the averaged trace from three repeated stimuli (solid) with SD (shadow) (right). **F**, Comparison of the integrated $\Delta F/F_0$ between nIRCats and GRAB_{DA3m}. The integrated $\Delta F/F_0$ was obtained from a 74 μ m $\times$ 74 μ m field of view. Each point is the average of three stimuli. $n = 6$ brain slices from 1 mouse. Data were fitted with linear regression (dashed line; $R^2 = 0.62$). **G**, Example of $\Delta F/F_0$ of GRAB_{DA3m} (top, green) and nIRCats (bottom, purple), integrated from a 74 μ m $\times$ 74 μ m field of view, in response to depolarization via potassium application (100 mM, 1 s). Individual response (left) and the averaged trace from three repeated stimuli (solid) with SD (shadow) (right). **H**, Comparison of the integrated $\Delta F/F_0$ between nIRCats and GRAB_{DA3m}. The integrated $\Delta F/F_0$ was obtained from a 74 μ m $\times$ 74 μ m field of view. Each point is the average of three stimuli. $n = 3$ brain slices from 1 mouse. Data were fitted with linear regression (dashed line; $R^2 = 0.78$).

dopamine release. Note GRAB_{DA3m} is insensitive to quinpirole because it is based on a D1 receptor scaffold and can thus reliably be used to measure dopamine modulation in conjunction with quinpirole (Zhuo et al., 2024). Quinpirole significantly reduced the nIRCats responses by $\sim 70\%$ in the number of identified ROIs and $\sim 60\%$ in the integrated $\Delta F/F_0$ (Fig. 4B–D). GRAB_{DA3m} exhibited no significant change in the number of ROIs or in the integrated $\Delta F/F_0$ (Fig. 4E,F), consistent with sensor saturation. These results highlight that the lower-affinity nIRCats sensor can spatially resolve localized dopamine hotspots, providing a useful tool for mapping high dopamine release microdomains and assessing how these patterns change with genetic manipulation (Black et al., 2025) or experimental condition (Mun et al., 2026).

Simultaneous two-photon calcium imaging and nIR dopamine imaging in the developing retina

To demonstrate the applicability of the microscope to other visible fluorophores and other neural circuits, we performed simultaneous two-photon calcium imaging with Cal520 and dopamine imaging with nIRCats in the retina. In the retina, the sole source of dopamine is a sparse population of amacrine cells which play essential roles in both retinal development (Reis et al., 2007; Liang et al., 2023) and adult light adaptation (Jackson et al., 2012; Roy and Field, 2019; Goel and Mangel, 2021). Although we previously demonstrated that spontaneous retinal waves evoke dopamine release (Arroyo and Feller, 2016), those experiments were limited by the use of one-photon, FRET-based CNiFER sensors (Müller et al., 2014) with relatively slow kinetics. As a result, dopamine could only be detected as a bulk signal with limited temporal resolution and no ability to resolve the spatial structure of dopamine release across the retinal tissue. Dopamine release has not been imaged directly from adult retina.

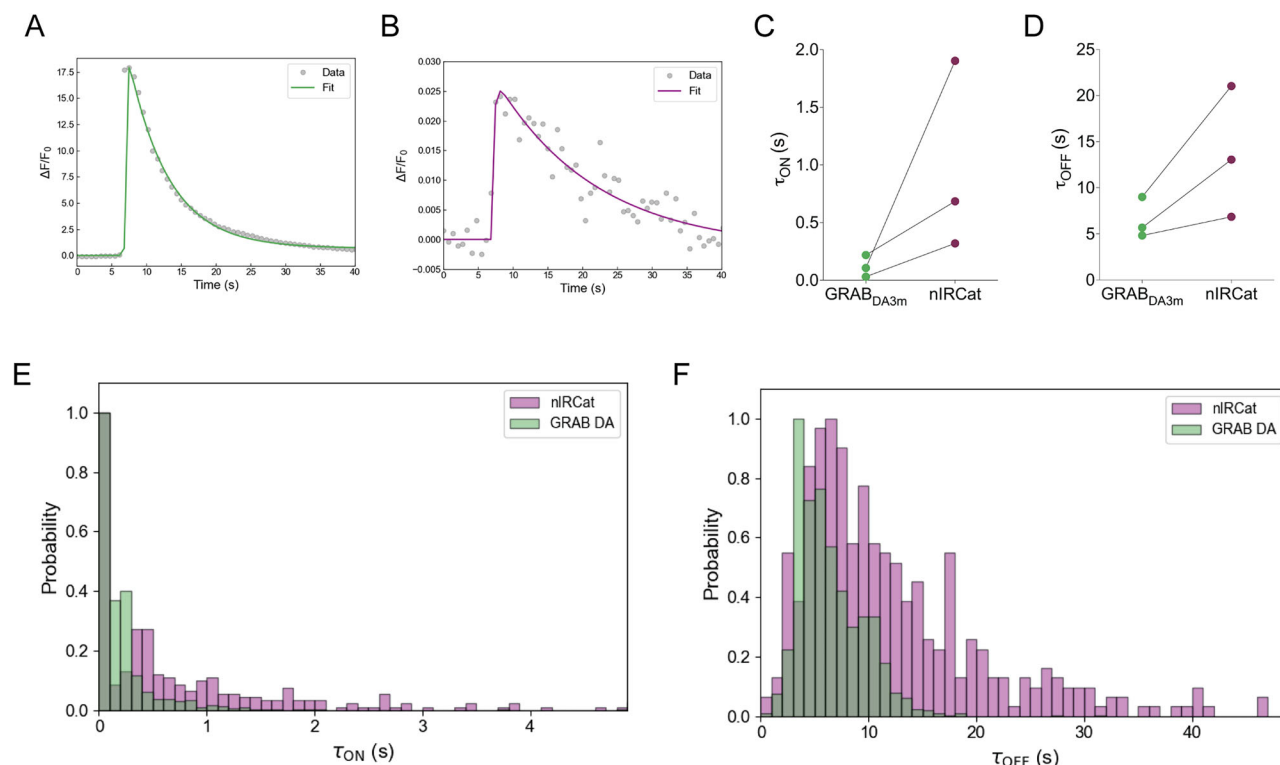


Figure 3. Comparison of response kinetics of nIRCat and GRAB_{DA3m} to evoked dopamine release. **A**, Example average responses of GRAB_{DA3m} to electrically evoked dopamine release in striatal slices and their fits used to obtain τ_{ON} and τ_{OFF} . **B**, Same as **A** for nIRCat. **C**, Summary data comparing τ_{ON} for GRAB_{DA3m} and nIRCat for responses averaged over the entire field of view (74 $\mu\text{m} \times 74 \mu\text{m}$). Each point is the average of three stimuli. $n = 3$ brain slices from 1 mouse. **D**, Same as **C** for τ_{OFF} . **E**, Distributions of τ_{ON} for GRAB_{DA3m} (green) and for nIRCat (pink) for local ROIs (6 $\mu\text{m} \times 6 \mu\text{m}$). **F**, Same as **E** for τ_{OFF} .

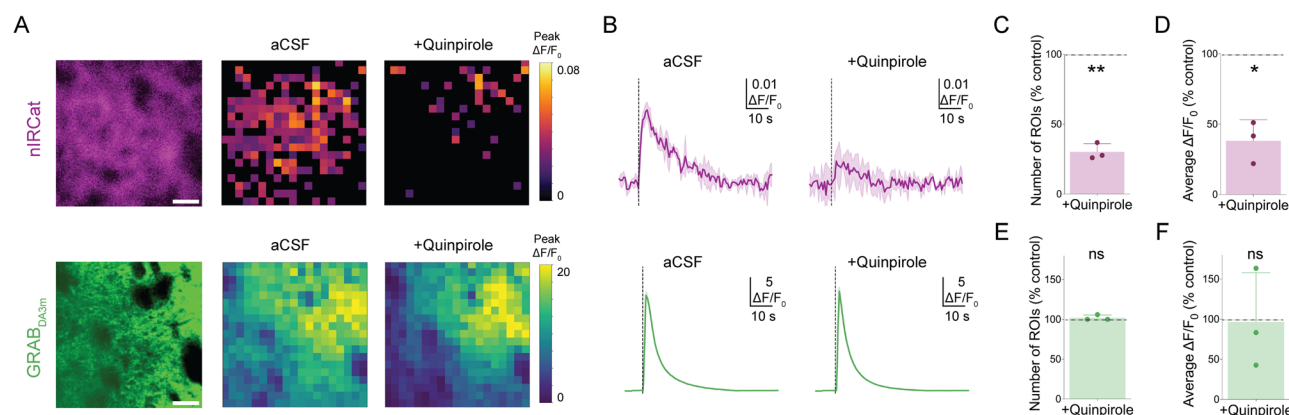


Figure 4. Use of nIRCat to identify localized high dopamine release sites. **A**, Left, Fluorescent signal from nIRCat (top) and GRAB_{DA3m} (bottom). Scale bar, 20 μm . Middle, Heat map of $\Delta F/F_0$ responses to 300 μA electrical stimulation in 6 $\mu\text{m} \times 6 \mu\text{m}$ ROIs in aCSF. Right, Heat map after 10 μM quinpirole application. **B**, Example of $\Delta F/F_0$ from the entire field of view. Average of $n = 3$ stimuli (solid) with SD (shadow). **C**, The percent of ROIs significantly above threshold after application of 10 μM quinpirole in the nIRCat channel. Each data point is the average of three stimuli. $n = 3$ brain slices from 1 mouse. $p = 0.0023$ by paired t test ($t_{(2)} = 20.68$). **D**, Same as **C** for average $\Delta F/F_0$ from the entire field of view (74 $\mu\text{m} \times 74 \mu\text{m}$) in the nIRCat channel. $p = 0.0188$ by paired t test ($t_{(2)} = 7.194$). **E**, Same as **C** for GRAB_{DA3m}. $p = 0.4226$ by paired t test. **F**, Same as **D** for GRAB_{DA3m}. $p = 0.9294$ by paired t test. * $p \leq 0.05$, ** $p \leq 0.01$, ns = not significant.

Using our dual-imaging system—which combines near-infrared detection of the dopamine nanosensor nIRCat with two-photon calcium imaging of Cal520—we directly visualized dopamine dynamics in P4–P8 retinas (Fig. 5). Brief K^+ puffs (100 mM, 1 s) evoked robust fractional fluorescence changes in both indicators (Fig. 5B), while there was no detectable change with ACSF application (Fig. 5D).

To assess dopamine release during retinal waves, we repeated these experiments under conditions of spontaneous activity only. Aligning nIRCat signals to spontaneous wave peaks revealed a strong temporal coupling between wave

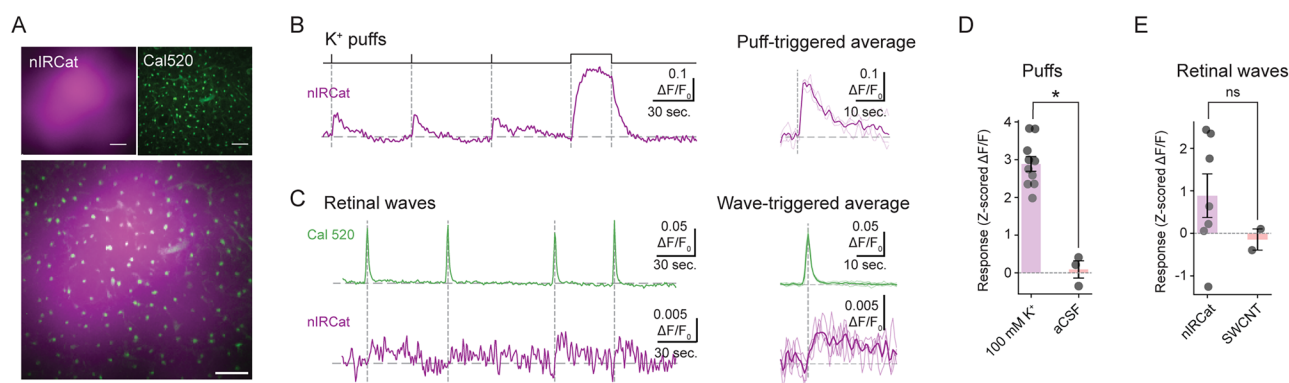


Figure 5. nIRCat response to dopamine release in retina. **A**, Pseudocolor images of Cal 520 (green) and nIRCat (purple) in the retina's inner plexiform layer. Scale bars are 20 μ m. **B**, Left, Fractional change in fluorescence of nIRCat in response to dopamine release evoked by applications of K^+ (100 mM). Pressure monitor above the traces indicates timing of puffs: three short (1 s) and one long (30 s) to probe the saturated response. Right, Puff-triggered average (thick line) and individual trials (thin lines) for the 1 s puffs. **C**, Left, Simultaneous imaging of Cal 520 (green) and nIRCat (purple). Vertical dashed lines mark the peaks of Ca^{2+} transients associated with spontaneous retinal waves. Right, Wave-triggered averages (thick lines) and signals from individual wave events (thin lines). **D**, Responses to puffs of 100 mM K^+ or aCSF (a negative control). Each point is an experimental replicate (a retina piece). $n = 7$ retina pieces from 4 mice for K^+ and $n = 3$ retina pieces from 3 mice for aCSF. Error bars represent the standard error of the mean. $p = 0.01190$ by Mann–Whitney U test ($t = 18.0$). **E**, Responses to retinal waves measured after loading with dopamine-sensitive near-infrared catecholamine sensor (nIRCat) or single-walled carbon nanotubes (SWCNT; a negative control). Each point is an experimental replicate (a retina piece). $n = 7$ retina pieces from 5 mice for nIRCat and $n = 2$ retina pieces from 1 mouse for SWCNT. Error bars represent the standard error of the mean. $p = 0.1667$ by Mann–Whitney U test ($t = 11.0$). $*p \leq 0.05$, ns = not significant.

activity and dopamine release (Fig. 5C). However, although nIRCat responded robustly to strong depolarization evoked by K^+ puffs, its lower dopamine affinity resulted in poor signal-to-noise for wave-evoked transients (Fig. 5E). Hence, the lower affinity of the nIRCat limits its ability to detect the lower dopamine concentrations associated with spontaneous retinal wave.

Discussion

We presented a straightforward approach to introduce a nIR detection capacity to an existing two-photon microscope. This was achieved by introducing a single-mode optical fiber prior to the nIR detector without requiring an additional excitation laser. The fiber-coupled design also provided confocal capability and minimized background nIR signals. This integrated approach allows real-time comparison of dopamine dynamics measured with complementary sensors, as well as simultaneous monitoring of neuronal depolarization and neuromodulator release in intact tissue.

Introducing an nIR channel enabled simultaneous, real-time imaging of visible and nIR fluorescence. Dual-channel imaging is a powerful strategy widely used within the visible spectrum—such as concurrent recording of green and red fluorescence—to reveal complex interactions across cellular and circuit processes (Zhuo et al., 2024). In this study, we extend this capability into the near-infrared range by simultaneously monitoring nIRCat fluorescence alongside dynamically reporting genetically encoded dopamine or calcium indicators in the visible channel. While previous studies have coimaged nIRCats with neurons labeled with visible fluorophores in culture (Bulumulla et al., 2022; Elizarova et al., 2022), our approach introduces two advances: (1) functional imaging in the visible channel rather than static structural labeling and (2) confocal capability in the nIR detection by implementing fiber-based coupling, which reduces background and improves optical sectioning for imaging in tissue. Although we focused on dopamine and calcium imaging, this platform is expandable to include additional sensors. For example, synthetic organic fluorophores with emission near 1,000 nm have been developed for structural imaging (Antaris et al., 2017). Furthermore, near-infrared calcium (Qian et al., 2019) and voltage indicators (Abdelfattah et al., 2023; Han et al., 2023) have been reported, although these probes emit at shorter wavelengths (~ 700 nm) and would require adjustments to the filter sets used in the current optical setup.

Multiplexed imaging of sensors with different affinities enables localization of dopamine release sites using a low-affinity sensor, while simultaneously measuring the spatial spread of physiologically relevant dopamine concentrations with high-affinity D1- or D2-based sensors. In addition, GRAB_{DA} is membrane-bound and therefore reports dopamine diffusion near the cell surface, whereas nIRCat is distributed throughout the extracellular volume, allowing measurement of the kinetics of bulk dopamine release. Together, these complementary sensors provide a unified framework for resolving both the spatial and temporal dynamics of dopamine signaling across cellular and tissue scales.

Our demonstration of simultaneous dopamine and calcium imaging in the retina showcased another potential niche for nIRCat applications, where the expression of genetically encoded sensors like GRAB_{DA} is technically challenging. Such contexts include tissues or organs where sensor expression is less characterized (e.g., retina, intestine), early developmental stages before robust expression can occur, or nontraditional model organisms lacking fully annotated genomes (e.g., meadow voles; (Mun et al., 2026)).

Live imaging of dopamine release in the retina would address several outstanding questions about how dopamine levels are regulated across development and adulthood (Roy and Field, 2019). Here we focused on early postnatal development, when dopamine is released in response to spontaneous retinal waves, which provide patterned activity essential for circuit refinement (Kirkby et al., 2013; Arroyo and Feller, 2016). Dopamine release in the retina is confined to a sparse population of amacrine cells, though recent findings indicate that retinal ganglion cells (RGCs) might transiently release dopamine in a manner that influences vascular development (Liang et al., 2023). Important open questions remain regarding the relative contributions of circadian influences versus melanopsin-expressing ipRGC-driven activity in regulating dopamine release (Zhang et al., 2008; Pérez-Fernández et al., 2019). Developing approaches for real-time dopamine imaging will therefore be essential for defining the sources, timing, and spatial dynamics of dopamine signaling during these critical stages of retinal maturation.

Although beyond the scope of this work, adapting this microscope for in vivo dual-color imaging would be an exciting next step, given the prevalence of two-photon microscopy in in vivo applications. Recent progress integrating nIRCats with fiber photometry (Klinger et al., 2025) suggests a promising direction, though several challenges remain: the signal-to-noise ratio would need to approach that of GRAB_{DA}, and nIRCats diffusion, biocompatibility, and clearance in brain tissue would require thorough characterization. Nonetheless, growing interest in near- and far-infrared imaging (e.g., nIR-III window; Feng et al., 2021) underscores the value of these technologies, and the approach described here provides a practical path toward longer-wavelength detection capabilities without constructing an entirely new microscope.

References

- Abdelfattah AS, et al. (2023) Sensitivity optimization of a rhodopsin-based fluorescent voltage indicator. *Neuron* 111:1547–1563.e9.
- Antaris AL, et al. (2017) A high quantum yield molecule-protein complex fluorophore for near-infrared II imaging. *Nat Commun* 8:15269.
- Arroyo DA, Feller MB (2016) Spatiotemporal features of retinal waves instruct the wiring of the visual circuitry. *Front Neural Circuits* 10:54.
- Bayer HM, Glimcher PW (2005) Midbrain dopamine neurons encode a quantitative reward prediction error signal. *Neuron* 47:129–141.
- Berridge CW, Waterhouse BD (2003) The locus coeruleus–noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain Res Rev* 42:33–84.
- Beyene AG, et al. (2019) Imaging striatal dopamine release using a nongenetically encoded near infrared fluorescent catecholamine nanosensor. *Sci Adv* 5:eaaw3108.
- Black AM, Komatsu N, Zhao J, Taskey SR, Serrano NS, Sharma R, Manoli DS, Landry MP, Beery AK (2025) Oxytocin receptors mediate social selectivity in prairie vole peer relationships. *Curr Biol* 35:4178–4187.e5.
- Blankenship AG, Ford KJ, Johnson J, Seal RP, Edwards RH, Copenhagen DR, Feller MB (2009) Synaptic and extrasynaptic factors governing glutamatergic retinal waves. *Neuron* 62:230–241.
- Bulumulla C, Krasley AT, Cristofori-Armstrong B, Valinsky WC, Walpita D, Ackerman D, Clapham DE, Beyene AG (2022) Visualizing synaptic dopamine efflux with a 2D composite nanofilm. *Elife* 11:e78773.
- Cambré S, Van Werveke W, Clercq MD, Erkens M, Martinati M, Wenseleers W (2025) Quantitative 2D fitting of fluorescence-excitation maps: excitation lineshape of single-wall carbon nanotubes. *Nanoscale Horiz* 10:3405–3415.
- da Silva JA, Tecuapetla F, Paixão V, Costa RM (2018) Dopamine neuron activity before action initiation gates and invigorates future movements. *Nature* 554:244–248.
- Del Bonis-O'Donnell JT, Mun J, Delevich K, Landry MP (2021) Synthetic nanosensors for imaging neuromodulators. *J Neurosci Methods* 363:109326.
- Elizarova S, Chouaib AA, Shaib A, Hill B, Mann F, Brose N, Kruss S, Daniel JA (2022) A fluorescent nanosensor paint detects dopamine release at axonal varicosities with high spatiotemporal resolution. *Proc Natl Acad Sci U S A* 119:e2202842119.
- Feng Z, et al. (2021) Perfecting and extending the near-infrared imaging window. *Light Sci Appl* 10:197.
- Goel M, Mangel SC (2021) Dopamine-mediated circadian and light/dark-adaptive modulation of chemical and electrical synapses in the outer retina. *Front Cell Neurosci* 15:647541.
- Han Y, et al. (2023) Bright and sensitive red voltage indicators for imaging action potentials in brain slices and pancreatic islets. *Sci Adv* 9:eadi4208.
- Jackson CR, Ruan G-X, Aseem F, Abey J, Gamble K, Stanwood G, Palmiter RD, Iuvone PM, McMahon DG (2012) Retinal dopamine mediates multiple dimensions of light-adapted vision. *J Neurosci* 32:9359.
- Kirkby LA, Sack GS, Firl A, Feller MB (2013) A role for correlated spontaneous activity in the assembly of neural circuits. *Neuron* 80:1129–1144.
- Klinger ME, Miller RA, Komatsu N, Shiu A, Wilbrecht L, Landry MP (2025) Optical fibers functionalized with single-walled carbon nanotubes for flexible fluorescent catecholamine detection. *Langmuir* 41:9654–9663.
- Lammel S, Hetzel A, Häckel O, Jones I, Liss B, Roeper J (2008) Unique properties of mesoprefrontal neurons within a dual mesocortico-limbic dopamine system. *Neuron* 57:760–773.
- Liang JH, Akhanov V, Ho A, Tawfik M, D'Souza SP, Cameron MA, Lang RA, Samuel MA (2023) Dopamine signaling from ganglion cells directs layer-specific angiogenesis in the retina. *Curr Biol* 33:3821–3834.e5.
- Müller A, Joseph V, Slesinger PA, Kleinfeld D (2014) Cell-based reporters reveal in vivo dynamics of dopamine and norepinephrine release in murine cortex. *Nat Methods* 11:1245–1252.
- Mun J, Power KC, Komatsu N, Tomatz SA, Beery AK, Landry MP (2026) Neurochemical imaging reveals changes in dopamine dynamics with photoperiod in a seasonally social vole species. *Proc Natl Acad Sci U S A* 123:e2509561123.
- Patriarchi T, et al. (2018) Ultrafast neuronal imaging of dopamine dynamics with designed genetically encoded sensors. *Science* 360:eaat4422.
- Pérez-Fernández V, Milosavljevic N, Allen AE, Vessey KA, Jobling AI, Fletcher EL, Breen PP, Morley JW, Cameron MA (2019) Rod photoreceptor activation alone defines the release of dopamine in the retina. *Curr Biol* 29:763–774.e5.
- Qian Y, et al. (2019) A genetically encoded near-infrared fluorescent calcium ion indicator. *Nat Methods* 16:171–174.
- Reis RA, Ventura AL, Kubrusly RC, de Mello MC, de Mello FG (2007) Dopaminergic signaling in the developing retina. *Brain Res Rev* 54:181–188.
- Roy S, Field GD (2019) Dopaminergic modulation of retinal processing from starlight to sunlight. *J Pharmacol Sci* 140:86–93.

- Sippy T, Tritsch NX (2023) Unraveling the dynamics of dopamine release and its actions on target cells. *Trends Neurosci* 46:228–239.
- Speranza L, di Porzio U, Viggiano D, de Donato A, Volpicelli F (2021) Dopamine: the neuromodulator of long-term synaptic plasticity, reward and movement control. *Cells* 10:735.
- Stosiek C, Garaschuk O, Holthoff K, Konnerth A (2003) In vivo two-photon calcium imaging of neuronal networks. *Proc Natl Acad Sci U S A* 100:7319–7324.
- Sun F, et al. (2018) A genetically encoded fluorescent sensor enables rapid and specific detection of dopamine in flies, fish, and mice. *Cell* 174:481–496.e19.
- Witkovsky P (2004) Dopamine and retinal function. *Doc Ophthalmol* 108:17–39.
- Yang SJ, Del Bonis-O'Donnell JT, Beyene AG, Landry MP (2021) Near-infrared catecholamine nanosensors for high spatio-temporal dopamine imaging. *Nat Protoc* 16:3026–3048.
- Zhang DQ, Wong KY, Sollars PJ, Berson DM, Pickard GE, McMahon DG (2008) Intraretinal signaling by ganglion cell photoreceptors to dopaminergic amacrine neurons. *Proc Natl Acad Sci U S A* 105:14181–14186.
- Zhuo Y, et al. (2024) Improved green and red GRAB sensors for monitoring dopaminergic activity in vivo. *Nat Methods* 21:680–691.