SCaMP: An Improved Red Fluorescent Calcium Indicator for In Vivo Imaging

Abhi Aggarwal*[1,2], Jeremy P. Hasseman*[1], Amelia Waring*[1], Jennifer Y. Li[3], William J. Wright[3], Xingyang Fu[4], Abigail E. Stuart[1], Kui Wang[5], Luke T. Coddington[1], Jihong Zheng[1], Brendan Power[1], Daniel Reep[1], Arthur Tsang[1], Getahun Tsegaye[1], GENIE Project Team[1], Ronak H. Patel[1], Yuanhua Wang[5], Zhengyuan Pan[5], Richard Ikegami[1], Sandeep Kumar[4], Daniel Bushey[1], Robert E. Campbell[6], Wyatt Korff[1], Yu Mu[5], Daniel A. Colón-Ramos[4], Joshua T. Dudman[1], Vivek Jayaraman[1], Takaki Komiyama[3], Kaspar Podgorski[7], Alison G. Tebo†[1], Glenn C. Turner†[1]

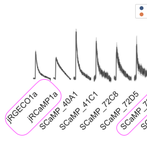
[1] HHMI Janelia Research Campus; [2] University of Calgary; [3] University of California San Diego; [4] Yale University; [5] Institute of Neuroscience, Chinese Academy of Sciences; [6] University of Tokyo; [7] Allen Institute for Brain Science *Co-first authors †Co-corresponding authors

Introduction

Red-shifted calcium indicators enable spectral multiplexing, allowing simultaneous measurement of activity across neural populations (e.g., GCaMP + red GECIs) or coupling calcium signals with neurotransmitter release (e.g., iGluSnFR + red GECIs).

While existing red GECIs perform well, they lag behind the jGCaMP8 family in key metrics. They also exhibit important limitations: jRGECO1a can undergo blue-light-induced photoswitching, complicating optogenetic experiments and, like other mApple-based sensors, accumulates in lysosomes as bright, non-responsive species, elevating background and compressing dynamic range.

To address these issues, we developed SCaMP, a red GECI based on mScarlet, one of the brightest red fluorescent proteins and one that does not photoswitch. SCaMP exhibits improved signal-to-noise and $\Delta F/F_0$ relative to prior red indicators, along with higher baseline brightness.



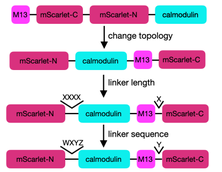
SCaMP: Red Calcium Indicator

- New red calcium indicator based on mScarlet - the brightest of the mRFPs
- Initial design work lead to a sensor with sensitivity and kinetics comparable to jRGECO.
- Further optimization via mutagenesis at 47 sites & pairwise combinations
- 3-5x higher SNR than jRGECO1a in cultured neurons

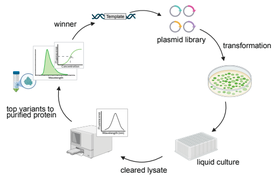
Initial Design

- Initial design work varied topology, linker length and linker sequence to get a calcium-sensitive starting sensor
- Directed evolution over 7 generations improved biochemical parameters of sensor (dynamic range, calcium affinity etc.) to be superior to existing red CaMPs
- End variant (72E5), tested in neurons (bottom), was re-named SCaMP v6 and advanced into the GENIE mutagenesis & screening pipeline

Initial Design



Directed Evolution

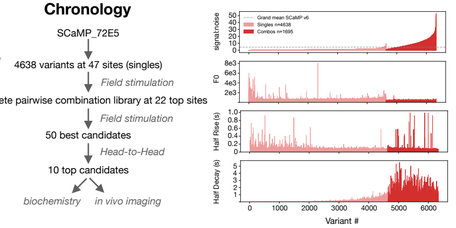


Optimization in Primary Neurons

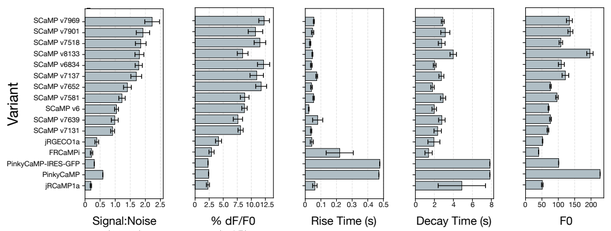
- 47 sites targeted 4,512 variants screened in primary neurons
- Top 11 sites (21 mutations) identified based on signal-to-noise
- All pairwise combinations constructed and evaluated in a secondary screen
- Top 10 variants compared head-to-head and rank-ordered for in vivo testing

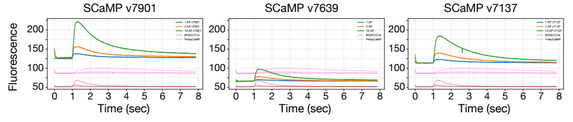
Site-Directed Mutagenesis

Chronology

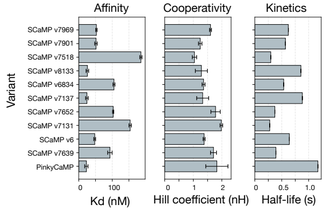


SCaMP vs Red GECIs

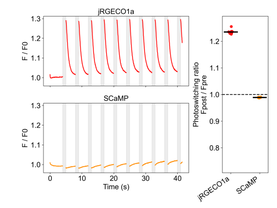




Biophysical Properties



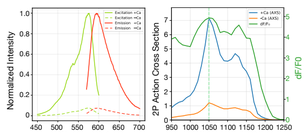
SCaMP variants span a range of calcium affinities; those below ~50 nM may preferentially report isolated spikes over trains. Hill coefficients indicate modest cooperativity, potentially supporting more linear rate coding. Calcium off-rates are generally faster than PinkyCaMP, with substantial variability across variants.



Photoswitching of jRGECO1a vs SCaMP. Purified protein (EGTA buffer) was imaged under 555 nm excitation with 1 s pulses of 475 nm light (15.8 mW/mm^2; shaded) to probe photoswitching. Effects were quantified as F_post/F_pre for each pulse (right).

	Max ΔF/F	Extinction Co. ε (M-1cm-1)		Quantum Yield ϕ		Lifetime τ (ns)	
		+Ca	-Ca	+Ca	-Ca	+Ca	-Ca
SCaMPv6	13	77660	75834	0.21	0.02	2.57	1.68
v7969	12	78797	75668	0.28	0.03	2.51	1.80
v7901	12	75061	76703	0.32	0.03	2.58	1.86
v7518	11	69909	76124	0.26	0.02	2.54	1.73
v8133	9	70213	74961	0.33	0.04	2.62	2.01
v6834	17	65991	74974	0.36	0.02	2.64	1.79
v7137	15	75980	76740	0.39	0.04	2.79	2.05
v7652	11	70614	74232	0.25	0.02	2.61	1.81
v7581	12	69750	75178	0.27	0.02	2.56	1.51
v7639	19	74652	76299	0.32	0.02	2.60	1.60
v7131	10	76025	77453	0.30	0.03	2.63	1.88
Pinky	26	66409	67108	0.46	0.02	2.78	1.47

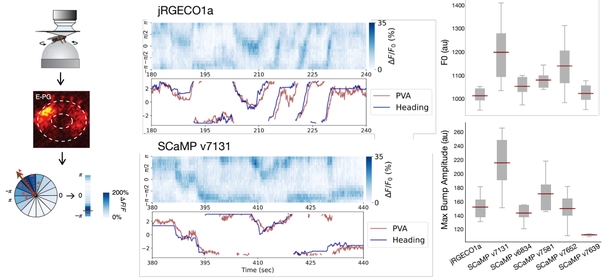
Biophysical properties of SCaMP variants.
Note: SCaMP variants exhibit calcium-dependent fluorescence lifetime shifts of ~ 0.8 ns



Left: 1P excitation/emission spectra of SCaMP (v7901, representative of others) peak at 575/595 nm.
Right: 2P action spectrum peaks at 1050 nm.

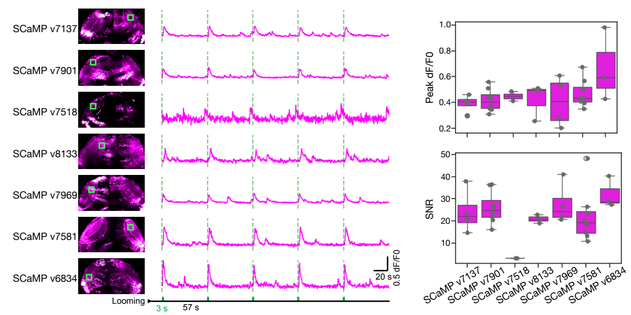
In vivo testing

Drosophila Head Direction Circuit



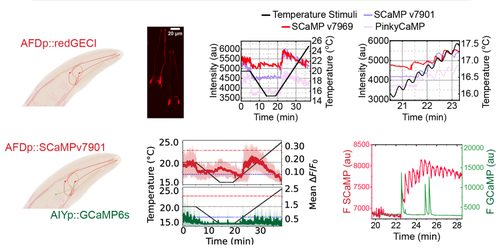
Imaging the head-direction circuit in Drosophila.
Left: schematic of a fly walking on a spherical treadmill during imaging. As the fly changes direction, a bump of activity in the ring-shaped ellipsoid body shifts accordingly.
Middle: jRGECO1a (top) and SCaMP v7131 (bottom) signals (blue heatmap) as the fly changes heading (red: actual heading; blue: heading predicted from bump position).
Right: quantification of baseline fluorescence and signal amplitude across different sensors.

Zebrafish Visual System



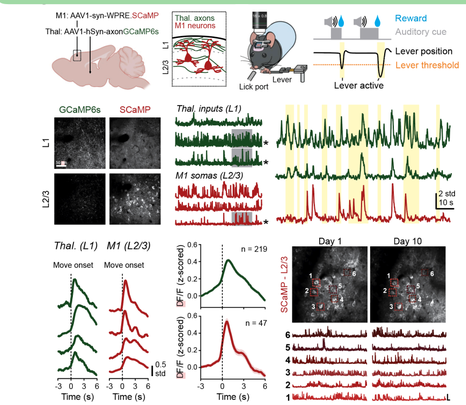
Testing SCaMP variants in zebrafish.
Top-performing SCaMP variants were screened using looming stimuli to probe visual responses.
Left: example fields of view for each variant.
Middle: fluorescence traces show robust, time-locked responses to looming stimuli.
Right: quantification of peak $\Delta F/F_0$ and SNR reveals differences in performance across variants.

Multiplexed Imaging Across a Synaptic Pair in *C. elegans*



Two-color imaging of AFD (SCaMP) and AIY (GCaMP) neurons in *C. elegans*.
Top left: schematic and 60x spinning-disk confocal image showing SCaMP expression in AFD thermosensory neurons.
Top right: full trace (middle) and zoomed view (right) comparing the two best-performing SCaMP variants with PinkyCaMP (colored traces; see legend) during slow temperature ramps with superimposed rapid fluctuations (black). Both indicators track rapid temperature changes with high temporal fidelity.
Bottom left: schematic of the experimental setup for dual-color imaging of presynaptic AFD and postsynaptic AIY.
Bottom middle: mean dF/F0 traces of simultaneously recorded AFD (red; SCaMP) and AIY (green; GCaMP) pairs (n = 20 animals).
Bottom right: example simultaneous AFD and AIY responses; AIY responses are probabilistic relative to AFD, consistent with variable transmission across the AFD→AIY synapse.

Multiplexed Imaging Across Connected Brain Areas in Mouse

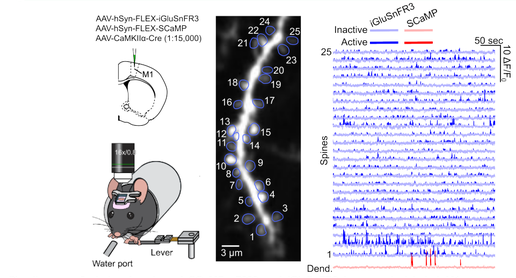


Simultaneous 2-photon imaging of thalamocortical inputs (GCaMP6s, green) and motor cortex (M1) neurons (SCaMP v7137, red) during a lever-pressing task.
Top: experimental schematic of dual-plane imaging in L1 (thalamic axons) and L2/3 (M1 neurons).
Middle: example field of view and calcium signals from thalamic inputs and M1 neurons.
Bottom: movement-aligned responses reveal coordinated activity across thalamic inputs and M1 neurons; stable expression enables longitudinal tracking of the same neurons across days of training

Population-level multiplexing of calcium activity and dopamine signals

Simultaneous fiber photometry of dopamine release (dLight) and neuronal activity (SCaMP variants) in dorsal striatum.
Top: experimental schematic and example fiber placement.
Middle: spontaneous activity (dF/F0; SCaMP, red; dLight, green).
Bottom: reward-aligned responses (t = 0; expanded timescale).
SCaMP dF/F0 amplitudes are modest (bottom left), but z-scoring - an explicitly signal-to-noise–normalized metric - reveals strong, time-locked responses (bottom right), indicating reliable detection despite small absolute dF/F0 changes.

Cellular-resolution multiplexing of calcium activity and glutamate signals



Simultaneous 2-photon imaging of SCaMP v7639 and iGluSnFR3 in sparsely labeled M1 neurons during a lever movement task. Glutamate release onto individual spines (numbered ROIs) is monitored alongside SCaMP signals from the dendritic trunk. Signals exceeding a threshold are highlighted in saturated colors (right), revealing coordinated synaptic and dendritic activity.

REAGENT DISTRIBUTION: Reagents will be distributed via Addgene upon selecting final variant & posting manuscript to bioRxiv

REFERENCES:
PinkyCaMP: Fink...Masseck https://doi.org/10.1038/s41592-026-03065-2
FRCaMPi: Zhou...Piatkevich https://doi.org/10.1371/journal.pbio.3003048
jRGECO1a: Dana...Kim https://doi.org/10.7554/eLife.12727

ACKNOWLEDGEMENTS: Many thanks to: Deepika Walpita & Phuong Nguyen (Janelia Cell Culture), Mike Perham (Scientific Operations) Invertebrate Shared Resource, Viral Tools, and Vivarium teams for expert support. Marius Pachitariu & Qingqing Zhang for help with in vivo imaging.
The GENIE Steering Committee is currently: Josh Dudman, Vivek Jayaraman, Wyatt Korff, Eric Schreiter, Hari Shroff, Alison Tebo