

A subdural CMOS optical device for bidirectional neural interfacing

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Section S1. ASIC design

The imager array is $5.1\text{ mm} \times 6.8\text{ mm}^2$ and is comprised of a 3×4 array of macros, each consisting of 14 blocks of 16×16 SPAD pixels with a pitch of $25\text{ }\mu\text{m}$, and 12.5% of the array is removed for μLED bond pads and drivers. The drivers for the fluorescence-exciting blue μLEDs are configured for continuous-wave (CW) illumination for high signal-to-noise-ratio (SNR) imaging whereas the drivers for the optogenetic red μLEDs are configured on a frame-by-frame basis allowing for pulsing durations in increments of 5 ms at 200 fps. Unlike an earlier design¹ in which detected photon counts are stored in shared-row counters, this design² supports 10-bit pixel-level counters and a global shutter, improving the ratio of integration time to frame period 100-fold from 0.625% to 99.6%, while supporting frame rates of up to 200 fps with a 100 MHz reference clock. Since the standard cell flip-flops consume more area and contain buffers for timing and driving large capacitive loads, a custom in-pixel flip-flop was designed to minimize area usage so that the 10b asynchronous ripple carry counter could be brought into the pixel. The chip's frame rate is 200fps, when reading the entire array, or 400fps, when reading half the array (128×192); the latter allows for the imaging of fast state-of-the-art voltage indicators³. Each pixel consists of two passively quenched SPADs whose outputs are combined into an in-pixel 10b counter. The data is read off the imager in a rolling column readout structure where the pixel data is buffered with up to 16 repeater cells throughout the array and loaded into a data serializer for streaming off chip. The data transmission module is a 1920b:1b shift register based serializer.

Section S2. Single Photon Avalanche Diodes

A single photon avalanche diode (SPAD) is a semiconductor p-n junction with a reverse-bias (V_{SPAD}) exceeding the breakdown voltage (V_{BD}) by an amount referred to as the excess voltage (V_E), $V_{SPAD} = V_{BD} + V_E$, such that a single photogenerated carrier initiates an avalanche current through impact-ionization. Upon triggering, the voltage across the SPAD is reduced below V_{BD} to quench the avalanche and subsequently returned to V_{SPAD} to detect another photon. SPADs have been explored in many biophotonics applications including endoscopic FLIM⁴, super-resolution microscopy⁵, NIROT⁶, and PET⁷.

SPAD variants and device structure

To determine the best available SPAD to use as our detector, a design-of-experiments (DOE) was performed with many SPAD variants. The SPADs in this array varied the guard ring structure (standard p-well, high-voltage p-well, virtual p-epi) as well as the depth of the implant region (p+, high-voltage p-body, p-well, high-voltage p-well). The breakdown voltage of each of these devices was determined by performing a current-voltage (IV) sweep using a B1500 Semiconductor Parameter Analyzer (Keysight, USA). PDP measurements were performed by sweeping the wavelength on a Digikr m CM110 monochromator fed into the 819D Series Spectralon integrating sphere, allowing for us to simultaneously measure the incident power illuminating the SPAD with a 1936-R single channel optical power meter (Newport Corporation, USA).

The photon detection probability is calculated as:

$$PDP = \frac{\left[\left(\frac{N_{SPAD}}{t_{int}} - DCR \right) \cdot E_{photon} \right]}{P_{Newport}} \cdot \frac{A_{Newport}}{A_{SPAD}}$$

where N_{SPAD} is the number of SPAD counts collected within an integration time t_{int} , DCR is dark count rate, E_{photon} is the energy of a photon, $P_{Newport}$ is the photodiode power, $A_{Newport}$ is the

cross sectional active area of the photodiode, and A_{SPAD} is the cross sectional active area of the SPAD. The selected SPAD structure is a P implant diffusion layer sitting in a deep N well optimized for longer wavelength sensitivities. The active diameter of each SPAD is $6\mu\text{m}$ and N well sharing between the two adjacent chamfered square SPADs, results in a total active area of approximately $72\mu\text{m}^2$ and a fill-factor (FF) of approximately 11.5%. Each quad of SPADs sits in an isolation ring to prevent substrate noise from increasing the dark count rate.

SPAD architectures: time-gated versus photon-counting

The simplest SPAD quenching circuit is given with a passive quench transistor. The bias transistor operates as a large resistor, discharging the anode of the SPAD until there is a photon-induced avalanche current triggering a pulse that can be registered as a count in the external circuitry. The quenching circuit is passive-quench and passive-reset (PQRC) with dead-time determined by the VQ bias on the quench transistor. An on chip, array shared 3b digital to analog converter (DAC) provides the VQ bias from 450mV to 1.07V in steps of 80mV.

In first implementations^{1,8}, we designed a custom SPAD-based image sensor to enable time-gated (TG) fluorescence imaging through single-photon counting. Time gating uses the design of a high-speed active-quench, active-reset pixel circuit, which necessitates the implementation of a rolling shutter, shared off-pixel counter in order to maintain a small pixel pitch. In gated operation, the imager is only activated after each excitation pulse. Gating the sensor provides additional background rejection of scattered excitation light. The efficacy of time-gating, however, is limited in practice by a) the turn-off time of the excitation pulse, b) the fluorescence lifetime, and c) the impulse-response function (IRF) of the SPAD detectors. These photogenerated carriers diffuse, or random walk, until they reach the avalanche region of the SPAD causing a much delayed detected photon count⁹. The minority carrier lifetime in silicon substrate¹⁰ is long compared to the

nanosecond timescales required during time-gating operation of the SPAD. The ineffectiveness of time-gating to do time-domain filtering of the blue excitation light result necessitates the development of high-quality optical filters.

SPAD imager performance equations

Fill factor (FF) is determined by (A_{SPAD} is the cross-sectional active area of the SPAD, and A_{pixel} is the cross-sectional area of the pixel):

$$FF = \frac{A_{SPAD}}{A_{pixel}}$$

Photon detection efficiency (PDE) is given by (PDP is the photon detection probability and FF is the fill factor):

$$PDE = PDP \cdot FF$$

Noise equivalent power (NEP) is the minimum signal intensity required for an SNR of 1 within a 1 Hz bandwidth and is given by (h is Planck's constant, ν is the frequency of the photon, DCR is dark count rate, PDE is the photon detection efficiency):

$$NEP = h\nu \cdot \frac{\sqrt{2 \cdot DCR}}{PDE}$$

Noise · Power Figure of Merit ($Noise$ is the square root of the dark count rate, $Power$ is the measured electrical power of the sensor, and R_p is the pixel rate):

$$FoM = \frac{Noise \times Power}{R_p} \times 10^9 [e^- \cdot nJ]$$

Pixel Rate (N_H is the number of columns, N_V is the number of rows, r_{VO} is the readout time overhead per row, r_{HO} is the readout time overhead per column):

$$R_p = N_H N_V (1 + r_{VO}) (1 + r_{HO}) f$$

SPAD Dark Count Rate and Hot Pixels

The SPAD dark count rate is measured by evaluating the detected counts over a 1s integration in an optical dark room where no visible photons are present. Hot pixels are defined as pixels that have average dark count rates above a standard deviation of the median and are typically caused by fabrication defects in the SPAD structure. The median dark count rate is calculated to be around 17Hz. The total hot pixel accounts for less than 2% of the array.

SPAD power consumption

The inherent low-noise performance of SPADs, a result of low DCR and zero read noise, enables low-power sensing which is important for implantable applications to limit tissue heating. Since the SPAD is an activity-based high-gain sensor, power consumption scales with light intensity, and is a function of the energy consumption of a single avalanche event¹¹ $E_{ava} = C_{SPAD} \times V_{DD} \times [V_{DD} + V_{BD}]$ given an excess bias voltage of V_{DD} and multiplied by the incident photon flux. This contrasts to photodiode imagers which rely on fixed amplification overhead in the pixel shared column readout amplifiers and analog to digital conversion. A figure-of-merit (FoM)¹² is defined at the ratio of the noise-power product to the pixel rate. The SPAD noise is defined by $\sqrt{DCR \times t_{int}}$ and the pixel rate is the product of the number of rows, number of columns, and sensor readout rate. Our SPAD image sensor consumes 18.5mW in the dark and 22mW at peak saturation light intensity. Our SPAD sensor shows comparable or better FoM performance than low-noise CMOS image sensors.

Sensor Dynamic Range

The dynamic range (DR)¹³ of the sensor as determined by the number of bits (Q) of the counter is given by

$$DR = 20 \times \log_{10} 2^Q$$

For the 10-bit per-pixel counters, the DR is 60dB which is important in fluorescence microscopy preparations since a large specular reflection occurs at the tissue surface and insufficient spectral filtering can result in image saturation.

Section S3. Flip-chip CMOS

To be compatible with a flip-chip bonding process, the chip wire bond pads modified via a re-tape out (RTO) to convert to flip chip bond pads via a change in the back end of line (BEOL). The wire bond BEOL stack up includes a passivation via hole between the top metal and the aluminum pad layer as well as a top passivation opening to expose the aluminum pad. The IO pads for digital and supplies are along one dimension of the chip, and there are additional support bump pads on the remaining three sides of the chip. This is an unconventional method of using the flip-chip BEOL methodology, since normally the bumps are equally spaced at high density across the entire die, to get low inductance signal and power IO. However, since the center of our die is an imager that needs to be kept open for sensing, there are only peripheral IO bumps, approximating that of a wire bond packaged die.

Section S4. Polyimide Flexible Printed Circuit Board

The polyimide PCB is a flexible interposer for routing the reference clock (100 MHz, for maximum frame rate operation of 200fps), the scan-chain configuration signals, the voltage supplies (VDD of 1.5V, DVDD of 1.8V, VLED of 2.5V, and VSPAD of 20.9V), and the raw data stream (**Section S5**) to a field-programmable gate array (FPGA) which interfaces with a host computer to store and process the data (**Fig. 2D**). The full stack up of the one-layer flex PCB includes coverlay, coverlay adhesive, copper (conduction layer), and polyimide for a total package thickness of 52 μ m (**Fig. S3**). Stiffener is present on one end of the board to form a 200- μ m-thick zero-insertion-force (ZIF) connector to the host board. The copper layer within this connection edge was finished with electroless-nickel-electroless-palladium-immersion-gold (ENEPIG). To accommodate the chip, there is a laser-cut opening in the polyimide substrate such that the face of the integrated circuit is exposed when bonded to the board's back side.

Section S5. Data Transfer Protocol

Data controller on the chip

The pixel has a dual-SPAD architecture where the outputs of two SPADs are summed into a shared 10b in pixel counter. The chip's controller determines the state machine of the imager core with the readout operation performed in four steps: 1) the thermometer encoded REP signal is asserted for three clock cycles to allow the repeaters throughout the array to buffer the data, 2) the one-hot read column signal is enabled for two clock cycles to pass the data through the in pixel output transmission gates onto the repeater bus, 3) the load signal is used to latch the data into the data transmission serializer flip flops, and 4) REP, COL, and LOAD get de-asserted and RST gets asserted for one clock cycle to clear the data in the pixel counter. The digital clock runs on a clock division by eight of the reference clocks to save power. The data is streamed off-chip at 100MHz using the signals CLK, FLAG, and DATA.

Data interface on the host board

The host board is responsible for all data acquisition, including capturing, streaming, and storing the data from SCOPE on a host computer. The interface with the data stream from the chip, the host board uses the XEM7010-A50 (Opal Kelly) integration module both to program the chip and to convert the data coming from the chip into USB 2.0 format for packet transfer back to the host computer. The XEM7010-A50 module supports 2.7Mb of on-chip FPGA block RAM, 512 MiB DDR3 memory banks, switching power supplies, a multiple-output phase-locked loop (PLL), and up to 120 input-output (IO) connections. FPGA configuration bits are generated and compiled in Vivado Design Suite (Xilinx). The top-level hardware design logic (HDL) is programmed using Verilog and contains the top-level state machine for communicating with both the chip and the FrontPanel HDL for communicating with the Opal Kelly FrontPanel software API.

Using this FPGA interface, the SCOPe ASIC is initialized and programmed using a scan chain, which is enabled at chip initialization and whenever the state configuration of the chip needs to be updated. Since the scan chain is a serialized shift register, the amount of time needed to program the chip depends on the number of configuration bits. Data transmission from the SCOPe ASIC is serialized at 100 MHz with three off-chip signals: DATA, CLK, and FLAG. DATA is the raw sensor data with a serialized stream out; CLK is used for synchronizing the transmitter and receiver clock, and FLAG is a logical enable signal used for determining the validity of the data. The effective data rate for this link is determined by the number of pixels, the bit depth per pixel, and the frame rate: $192 \times 256 \times 10b \times 200Hz = 98.3Mb/s$. For the USB2.0 protocol, the Opal Kelly converts this serialized data stream into a 16b wide bus for use with the USB 2.0 pipe out. The conversion is done in two steps using Xilinx's intellectual property (IP) FIFO Generator with a pass-through 1:8 FIFO followed by an 8:16 FIFO. The pass-through FIFO converts the serialized data stream into an 8b parallel bus and operates on the chip CLK domain. The second FIFO buffers the data as it is streamed to the host computer and operates on the clock domain of the USB 2.0 interface (48 MHz). Chaining FIFOs in this manner works only if the secondary clock is faster than the data clock, such that the FIFOs do not fill. The data is read little-Endian off the chip and bit-reversed prior to sending over USB to simplify data visualization. The data is stored in binary files on the host computer and is read and reformatted into images with 10b precision in MATLAB.

Section S6. Die Thinning and Mechanical Flexibility

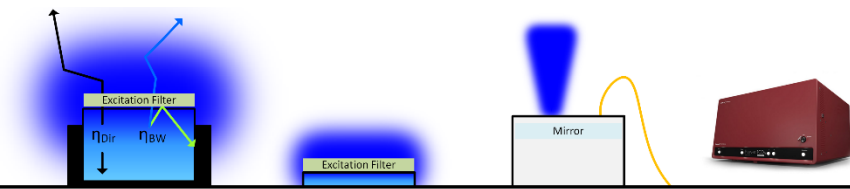
Once thinned, the bending stress in the CMOS die can be modeled using thin plate theory since the thickness of the CMOS die is orders of magnitude smaller than the lateral dimensions¹⁴. In this case, the minimum radius of curvature (R_{min}) before the stresses in the CMOS die exceed the maximum bending stress (σ_{max}), resulting in fracture, is related to the biaxial Young's Modulus, the Poisson's ratio (ν), and the thickness of the substrate by:

$$R_{min} = \frac{E \times t}{(1 - \nu)2\sigma_{max}}$$

For a Young's Modulus (E) of 85 GPa, Poisson's ratio (ν) of 0.21, and a substrate thickness of 15 μm , the minimum radius of curvature (R_{min}) is 4mm, assuming a maximum stress of 200 MPa¹⁵ for bulk silicon. In practice, because the CMOS die with interference filter is a more complex materials stack and defects created by backside thinning become nucleation site for fracture, the R_{min} is closer to 12.5mm.

Section S7. Comparison of integrated light sources

Coherent benchtop lasers have traditionally been utilized in conjunction with fiber-optic bundles¹⁶, tapered optical fibers¹⁷, or nanophotonic silicon probes^{18,19} to provide narrow spectral linewidths; however, they are too bulky to be implanted and have poor wall-plug efficiencies. Examples of integrated light sources include vertical-cavity surface-emitting lasers (VCSELs), organic μ LEDs (OLEDs), and solid-state μ LEDs. VCSELs provide coherent and narrowband light emission but suffer from complicated packaging due to wire bonding of a topside contact as well as poor electro-optic efficiency. OLEDs²⁰⁻²² provide an extremely scalable and ultrathin form-factor; however, they have poor electro-optic efficiency²² resulting in optical power densities of less than 0.2 mW mm^{-2} at 5V and suffer from spectral linewidths²³ in excess of 77-nm. The efficiency of the light source is given by the cascade of spectral (η_{BW}), electro-optic (η_{EO}), and directional (η_{Dir}) efficiencies. Flip-chip InGaN LEDs and OLEDs have relatively wide linewidths necessitating the use of excitation filters. Flip-chip InGaN μ LEDs have the best electro-optic efficiency compared with OLEDs and VCSELs. Combining these results, the flip-chip InGaN LEDs have relatively the best efficiency, additionally with the ease of packaging of not having to wirebond and encapsulate.



	Ideal Spec	InGaN LEDs	OLEDs	VCSELs	Laser
Integration	Flip-Chip / Fab	Flip-Chip	NanoFab	Wirebond	Wall-Plug
λ/BW	470/10nm	475/50nm	460/100nm	434/10nm	Supercontinuum
η_{BW}	100%	~50%	~10%	~90%	<1%
η_{EO}	100%	~40%	~5%	<1%	<1%
η_{Dir}	100%	~50%	~50%	~90%	~90%
η_{tot}	100%	10%	0.25%	<0.8%	<0.01%

Section S8. Modeling of fluorescent scene

Modeling light propagation in tissue

We estimate the light propagation in tissue using a combination of analytical solution and Monte Carlo solver²⁴ modeling the light path geometry. The analysis starts from the detector since the sensitivity of the pixel sensor informs the required μ LED light excitation. For a chief-ray angle (CRA), θ , of the SPAD pixel photodetector, the free-space path loss of an isotropically emitting fluorescent disk scene (**Fig. S10**) with radius of $R = d \tan(\theta)$ is given by the equation: $\frac{I_R}{I_0} = 0.5 \times \left[1 - \frac{d}{\sqrt{R^2 + d^2}} \right]$. With a CRA of 70° , the total normalized radiated emission from the fluorescent disk captured by the sensor pixel is 0.327. The Monte Carlo simulation uses a modified absorption coefficient of $\mu_a = 0.21 \text{ mm}^{-1}$ to model the amount of absorbed blue light due to the presence of a dense fluorescence volume. At a tissue depth of $200 \mu\text{m}$, the μ LED diagonal spacing of 0.6 mm (**Fig. 3B**) produces an illumination intensity at the mid-point between four μ LEDs that is 75% of the normalized μ LED power density (mW/mm^2) as a result of Lambertian emission, angular spreading, tissue scattering, and absorption. The normalized fluence near to the tissue surface is greater than one due to both backscattered light and internal reflection at the tissue-air interface²⁵. The diffuse optical property of the tissue causes the blue excitation light to scatter back towards the CMOS sensor at approximately 7.5% of the normalized illumination intensity.

Fluorescent conversion efficiency

We use literature values for the biophysical properties of the cells to determine the conversion from blue light excitation to green fluorescence emission. First, we determine the amount of spectral overlap, $S_{B \rightarrow gCaMP6f} = 0.6$, between the blue LED emission and the gCaMP6f absorption²⁶ (**Fig. S13**). In this fluorescent disk, the neuronal cellular density available for fluorescence tagging in the mouse visual cortex is approximately 96% (84% neuropil, 12% somas,

and 4% blood vessels)²⁷. Over-expression of gCaMP6f can result in damaging side-effects, so moderate to high expression can result in a total overall expression of 45% of all cells²⁸. Combining the cellular density with high expression results in an overall fluorescence of $D_n = 43.2\%$ per unit volume of tissue. At high expressions, the neocortical intracellular gCaMP6f concentration, c , in transgenic mouse models is 2-10 fold lower than typical conditions of AAV infections resulting in an intracellular concentration on the order of $c = 40\mu M$ ²⁸. The ratio of absorbed blue excitation light is then determined by the extinction coefficient²⁹, $\epsilon_c = 5030 M^{-1}mm^{-1}$ and application of the Beer-Lambert formula: $A = [1 - e^{-S_{B \rightarrow gCaMP6f} \times \epsilon_c \times c \times D_n \times t}]$ resulting in $A = 0.5\%$ for a volumetric slice of cortical tissue with $t = 100\mu m$. The amount of green fluorescence signal generated by this fluorescence disk includes the fluorophore quantum yield²⁹, $Q_Y = 0.59$, resulting in a total blue-to-green conversion efficiency, $\eta_{Blue \rightarrow Green} = A \times Q_Y = 0.003$. Cascading the loss due to light path propagation and biophysical blue-green light transduction results in an overall end-to-end fluorescence signal detection chain efficiency given by the following equation:

$$\frac{I_{Green}}{I_{Blue}} = \eta_{GreenLightPath} \times \eta_{Blue \rightarrow Green} \times \eta_{BlueLightPath} = 0.327 \times 0.003 \times 0.75 = 0.00074$$

Section S9. Filtering requirements

Given the 60-dB DR of the sensors, the required optical filter is determined by SNR performance requirements. Noise is dominated by the photon-shot noise of the unrejected backscattered illumination light. SNR equation (P_{sig} is the signal power in photoelectrons, and B is the background power in photoelectrons):

$$SNR = \frac{P_{sig}}{\sqrt{P_{sig} + B}}$$

For simplicity, the received photons by the SPAD, I , assuming direct coupling can be determined by the number of photons per second per area leaving the LED (light power density normalized by the energy of a photon at 520nm), the cross-sectional area of the SPAD pixel, the photon detection efficiency, and t_{int} the integration time per frame.

$$I = \frac{\left[\frac{P_{LED} \left[\frac{W}{mm^2} \right]}{E_{ph}[J]} \right] \times A_{pixel}[mm^2] \times PDE \times t_{int}}$$

Signal power is determined by the blue to green fluorescence conversion efficiency and I :

$$P_{sig} = 0.00074 \times I$$

Background power is determined by the backscattering of the excitation light towards the sensor (Methods), I , and the optical density, OD , provided by the spectral filter stack:

$$B = 0.075 \times I \times 10^{-OD}$$

The signal and background powers are limited to the dynamic range of the sensor, which must fall between 0 and 1023 counts for a single frame. This constraint allows us to solve for I as a function of received sensor counts N_c :

$$P_{sig} + B = 0.00074 \times I + 0.075 \times I \times 10^{-OD} = N_c \in [0, 1023]$$

$$I = \frac{N_c}{0.00074 + 0.075 \times 10^{-OD}}$$

Finally, this result gets plugged into SNR equation and can be plotted for various values of N_c and

OD (**Fig. S11**):

$$SNR = \frac{0.00074 \times I}{\sqrt{0.00074 \times I + 0.075 \times I \times 10^{-OD}}}$$

Section S10. Computational Imaging

Texas two-step amplitude mask

Due to mask separability, the local spatially varying point-spread function (PSF) can be fully represented by a superposition of orthogonal terms (**Fig. S16**), where P_{od} and P_{cd} operate on the rows of X_d , and Q_{od} and Q_{cd} operate on the columns of X_d (the subscripts o, c, and d refer to “open”, “coded”, and “depth”, respectively). As a result, we can capture from a single shot, Y , of a superposition of 2D scenes, X_d , which can be computationally “refocused” to retrieve each of the depths.

$$Y = \sum_{d=1}^D (P_{od}X_dQ_{od}^T + P_{cd}X_dQ_{cd}^T)$$

With the calibration matrices $P_{od,cd}$ and $Q_{od,cd}$ and a raw image sensor capture, Y , the scene is computationally reconstructed (**Fig. S17**), by solving a regularized least-squares problem where M is a 192×256 binary matrix representing the μ LED blocks and $\odot$ is the Hadamard product.

$$Y = M \odot (P_o X Q_o^T + P_c X Q_c^T)$$

Unlike a conventional image sensor, our imager is missing 12.5% of the pixels due to the embedded μ LEDs. The reconstruction accounts for these missing sensor pixels during reconstruction. While we were able to achieve good results using high-contrast scenes such as fluorescent beads and double line slits, the amplitude mask is not optimized to work with low contrast biological scenes. Future implementations can head towards phase masks with optimized PSF contours and high-contrast features to provide good protection against noise amplification in these low-contrast scenes and in scattering media similar to epifluorescence imaging³⁰; nevertheless, improvements must be made to further reduce the working distances to allow for fully implantable operation.

Section S11. *In-vivo* experimental discussion

Optical crosstalk with ChRmine.

The ChRmine activation spectrum is very-wide band allowing for optogenetic stimulation with a variety of wavelengths (**Fig. S23**). Given that 80% of the blue LED light directly contributes to induced photocurrent, the light power density used for gCaMP6f imaging needs to be maintained below the threshold for robust neural activation ($< 0.1 \text{ mW/mm}^2$)³¹. The light propagation losses mean that the blue excitation light falls below ($< 0.09 \text{ mW/mm}^2$) the stimulation threshold for neurons located in the tissue. For one photon bidirectional optical experiments, the optical crosstalk can be further reduced by shifting to further, red-shifted opsins such as Chrimson³².

Mesoscopic versus single cell.

Cellular imaging using two-photon microscopy provides robust recordings of single neuron resolution activity with FoVs restricted to a few hundred microns. Alternatively, mesoscopic imaging is a rapidly growing widefield single-photon fluorescence imaging approach that provides extremely large multiple millimeter FoVs at high spatiotemporal resolution. Thus, mesoscopic imaging provides a powerful tool to investigate largescale neuronal networks spanning multiple brain regions at high resolution. One limitation of mesoscopic imaging is that the measured fluorescence signal reported from the superficial layer of the cortex is a weighted integration of neuronal signals by depth³³, and that the summed signal include contributions from cell somata and neuropil^{34,35}. Another limitation is the overlap of green fluorescence with the endogenous activity-dependent absorption caused by blood volume and oxygenation. Typically, a separate green LED light source is used in reflectance mode to measure these hemodynamic signals to subtract them from the fluorescence video capture. However, the contribution of blood volume in GCaMP mice is relatively low compared to the larger activity-dependent fluorescence signals^{36,37}.

Section S12. Power dissipation and heating

While heating is usually disregarded in the development of conventional miniscopes due to the thermal isolation of the heat source (μ LEDs and CMOS image sensor) from the tissue, this is not appropriate for fully implanted devices. The design of a custom low-power integrated circuit allows us to limit the extraneous power consumption found in commercial general-purpose CMOS imaging systems, as well as to engineer the sensor front end for low-light biological scenes by using SPADs, as described earlier. In addition, the proximity of the optical device to the tissue interface allows for efficient optical interrogation with the biological scene without the efficiency losses of lenses and apertures. In our *in vivo* experiments, SCOPE achieves *in-vivo* imaging at 40 frames per second (fps) with a power consumption of less than 10 mW for the sensor and $<110 \mu\text{W}$ per μLED , of which approximately $10 \mu\text{W}$ is the optical emission. (**Fig. S8** and **Table S5**). This contrasts with miniature mesoscopes³⁸ with similar FoVs and resolution which consume 226 mW in the image sensor and 31mW in the excitation LEDs. This translates to more than $13\times$ less power consumption from our illumination μLEDs and $22\times$ less power consumption from our image sensor. The heating of the device with an $200\text{-}\mu\text{m}$ -thick overlaid cortical slice was characterized using a FLIR infrared camera (see Methods) to be less than 1°C ³⁹ up to electrical powers of 20mW and optical powers of 1.2mW, which is approximately two orders of magnitude larger than needed to image and stimulate neural activity (**Fig. S24**).

Section S13. NHP Physiological Artifact Removal

Initial analysis of the activity revealed prominent physiological artifacts. First is a dominant low-frequency oscillation at 0.1-Hz associated with blood volume or oxygenation^{40,41}. Blood oxygenation is expected to have minimal contribution to contamination of the fluorescence signal due to the small differences in absorption between oxyhemoglobin and deoxyhemoglobin in the green wavelengths⁴². On the other hand, increases in blood volume can cause a slight decrease in both the blue excitation and green emission light. One way to correct blood volume is to employ a multi-wavelength strategy by measuring the ratio of green light reflectance to blue light reflectance and subtracting from the raw data to reduce the blood volume hemodynamic response. However, in practice, the activity dependent GCaMP signals are large enough to overcome the contribution of blood volume to fluorescence signals (<10%)^{36,40}. The strongest mixing of GCaMP neural signals with hemodynamics are in the 0-0.4 Hz frequency band⁴³. In cases without dual-wavelength hemodynamic correction, the GCaMP signals can be extracted by employing temporal high-pass digital filters with 3dB cutoffs at either 0.1 Hz^{38,44} or 0.5 Hz⁴⁵⁻⁴⁷ since most of the mesoscale functional imaging power is located in the delta band (0.1-4Hz). Other types of non-physiological artifacts include variations in mechanical coupling due to movements. Nevertheless, motion artifacts present in miniscope configurations associated with relative movement of the sensor and the brain⁴⁸ do not occur with the SCOPe device because it is in direct contact with the cortical surface. Moreover, in these recordings, the nonhuman primate was head-fixed which further reduced the coupling of motion artifacts into the sensor. In addition to low frequency physiological artifacts, there also exists a 3-Hz physiological oscillation due to the heart beat⁴⁹. We suppressed both of these low and high frequency artifacts by sequentially applying a high-pass

(with a cut-off of 0.5-Hz) and smoothing with a moving average filter of 10 samples (cut-off of 2-Hz).

Section S14. Nonhuman primate experiments - Feature decoder

Motor brain machine interfaces (BMI) consist of three main parts: a sensor to provide robust recordings of neural signals, a feature decoder to process the neural signal and predict a response or intention, and a prosthetic to restore lost functionality⁵⁰. Adaptive algorithms to track changes in recorded signals and predicted intent can also play a significant role. Here, we demonstrate using recordings during awake behavior the ability to deliver accurate recordings of optical neural signals in the motor cortex. To further validate the application of the SCOPe device to BMI, we developed a feature decoder that predicts movement intent from processed neural signals obtained by the SCOPe sensor. The decoder trained a ridge regression function that took as input predictor data a single frame of neural activity imaged by the device and returned as output the response data, a movement speed prediction. y denotes the response data specified as a n-by-1 vector, X denotes the predictor data specified as an n-by-p matrix, k is the ridge parameter that regularizes the regression function, and I is the identity matrix. The resulting coefficient estimator matrix, $\hat{\beta}$, is of dimension (p+1)-by-1.

$$\hat{\beta} = (X^T X + kI)^{-1} X^T y$$

The predicted response data $y_p(t)$ is a n-by-1 vector determined by the summation of where β_0 is the first coefficient estimate, β_j are the remaining coefficient estimates (2 to p+1), and $X_{t'}$ is the testing data specified as an m-by-p matrix at time t'.

$$y_p(t) = \beta_0 + X_{t'} \beta_j$$

The premotor dorsal and motor cortex have been studied for encoding of kinematic parameters such as direction^{51,52}, position⁵³, speed, and velocity⁵⁴. While direction is most strongly encoded in single unit activity, speed is most strongly encoded in high gamma local field potentials⁵⁵. The mesoscopic calcium fluorescence imaging modality (**Section S11**) employed in our system is able

to resolve multiunit activity, but not single unit spiking events. We, therefore, focused on decoding movement speed. Since the motor cortices predict movements that occur in the future, we varied the relative time of t and t' . To quantify prediction performance, we measured the correlation coefficients for the predicted speed from the feature decoder and the ground truth speed measured via behavior.

$$\rho(y, y_p) = \frac{\text{cov}(y, y_p)}{\sigma_y \sigma_{y_p}}$$

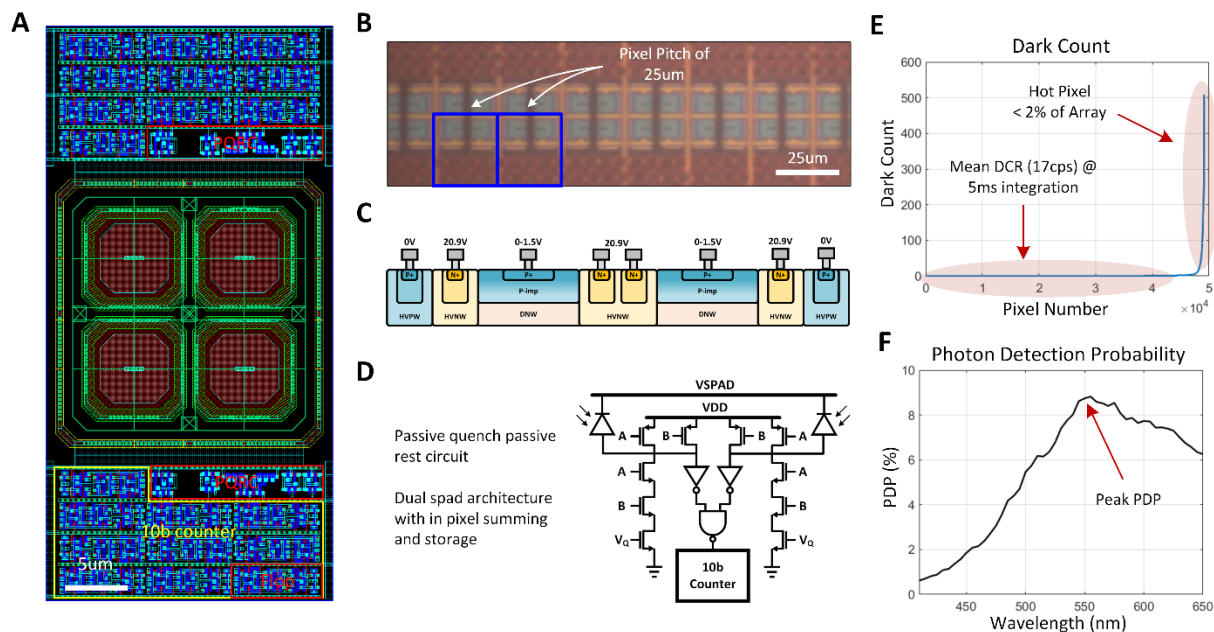


Fig. S1. SPAD characterization. (A) The layout of a 2×1 SPAD pixel with the top two SPADs summing into the top pixel 10b counter and the bottom two SPADs summing into the bottom pixel 10b counter. (B) Micrograph of a row of pixels and a pixel pitch of 25um. (C) Cross section of the SPAD well structure (as seen from the red line in a). (D) The quenching circuit is passive-quench and passive-reset (PQRC) with dead-time determined by the VQ bias on the quench transistor. (E) The median dark count rate is calculated to be around 17Hz. The total hot pixel accounts for less than 2% of the array. (F) The SPAD peak photon detection probability (PDP) is 9% at 550nm and an excess bias voltage of 1.5V.

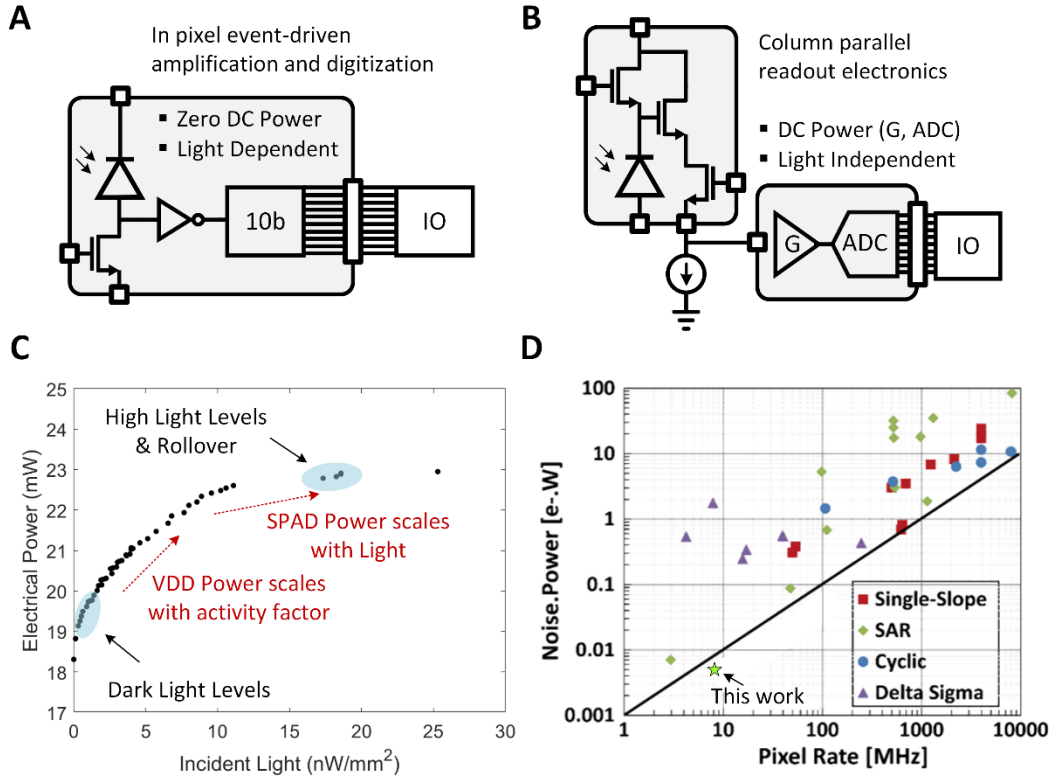


Fig. S2. SPAD power consumption. (A) Since the SPAD is an activity-based high-gain sensor, power consumption scales with light intensity. This contrasts to (B) photodiode imagers which rely on fixed amplification overhead in the pixel shared column readout amplifiers and analog to digital conversion. (C) Our SPAD image sensor consumes 18.5mW in the dark and 22mW at peak saturation light intensity. (D) CIS- and SPAD-based analog-to-digital signal acquisition is compared at light level of $2 \text{ nW}/\text{mm}^2$ by plotting the product of noise · power on the y-axis and pixel rate on the x-axis.

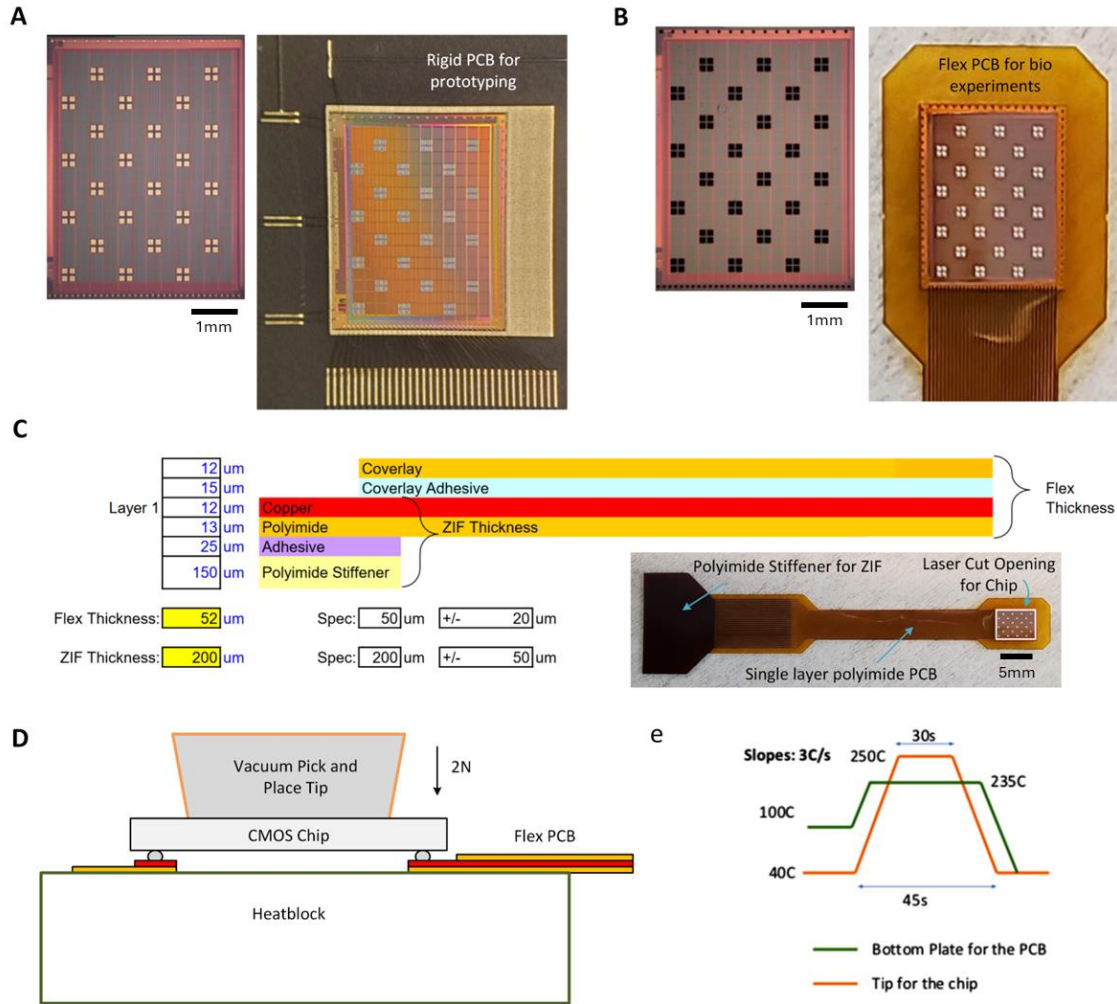


Fig. S3. Flip-chip bonding to flexible substrates. (A) Wirebond variant of back-end-of-line (BEOL) CMOS fabrication flow used for electrical and optical prototyping and characterization. The pads are wirebonded to an Electroless Nickel Electroless Palladium Immersion Gold (ENEPIG) surface-finish rigid PCB. (B) Flip chip variant with SnPb solder alloy bumps with 70μm bump diameter and 170μm pitch. (C) Flex PCB is designed for minimum thickness from the specified board house vendor for a single layer polyimide flex PCB. The total flex thickness is 52μm and a polyimide stiffener is added to the connector end of the flex cable for a total ZIF thickness of 200μm. There is a laser cut opening in the flex PCB to leave an optical window for the sensor to directly contact the tissue. (D) Cross section view of the flip chip bonding process

using Finetech FINEPLACER lambda for precision die attach to the flex PCB vacuum mounted onto the bottom plate heat block. A force of 2N is applied during the application of a heating program to allow for bump reflow without shorting between adjacent pads. (E) The heating program shows the temperature profiles for the bottom plate (green line) and the pick and place tip (orange trace).

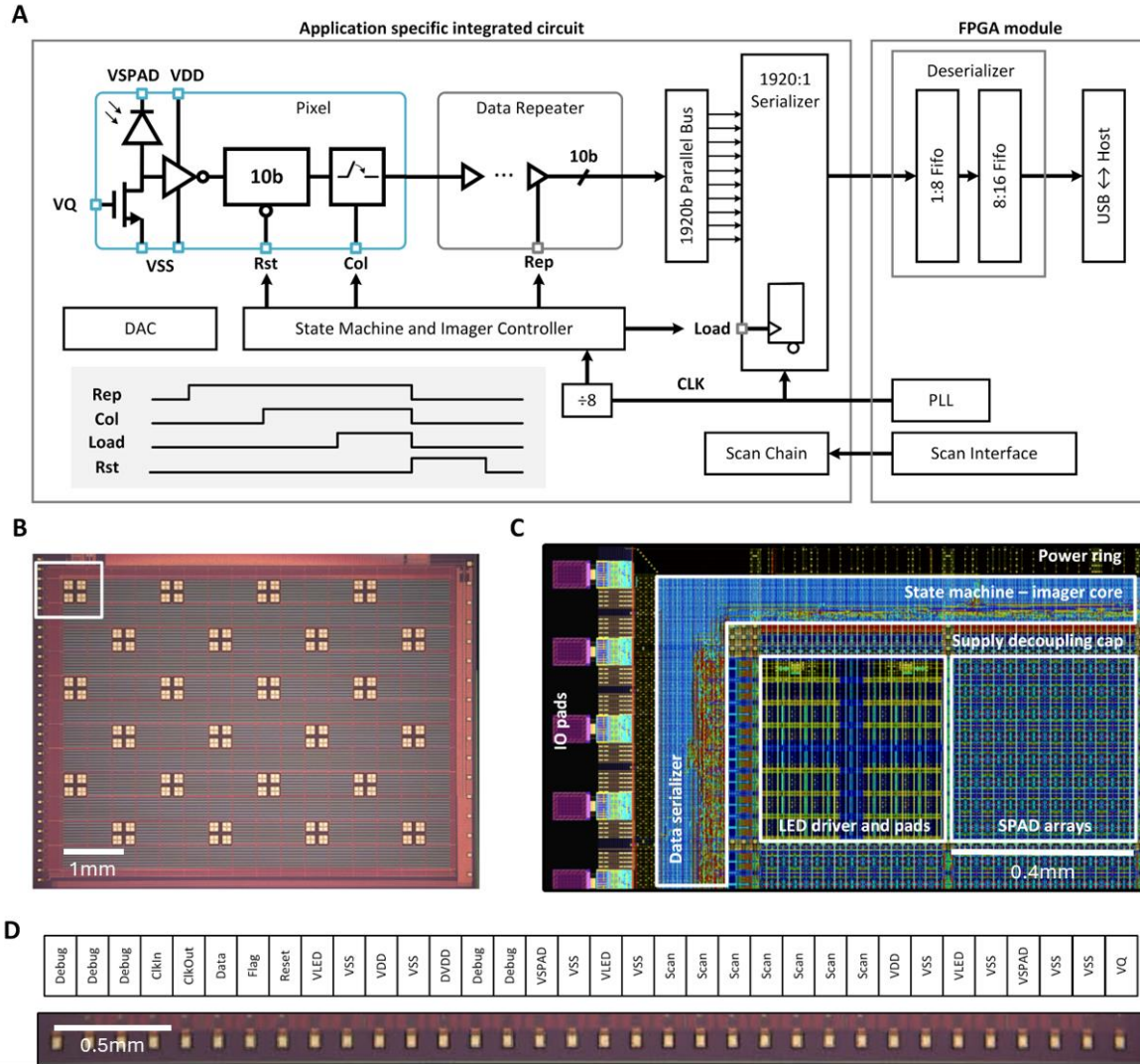


Fig. S4. Detailed overview of the CMOS system architecture. (A) The system on chip architecture consists of an analog 192×256 SPAD pixel array which interfaces with a digital imager core, data transmission block, and scan chain IO configuration. (B) Die photo of the fabricated chip. (C) Computer aided design layout (of white rectangle from B) highlighting the analog imager SPAD arrays, the digital core controller containing the imager core and data serializer, the power ring, and the IO pads. (D) A detailed view of the chip's IO and power signals resulting in a total of 34 pads. Many of the supplies are redundant to reduce the IR drop and minimize inductance and noise.

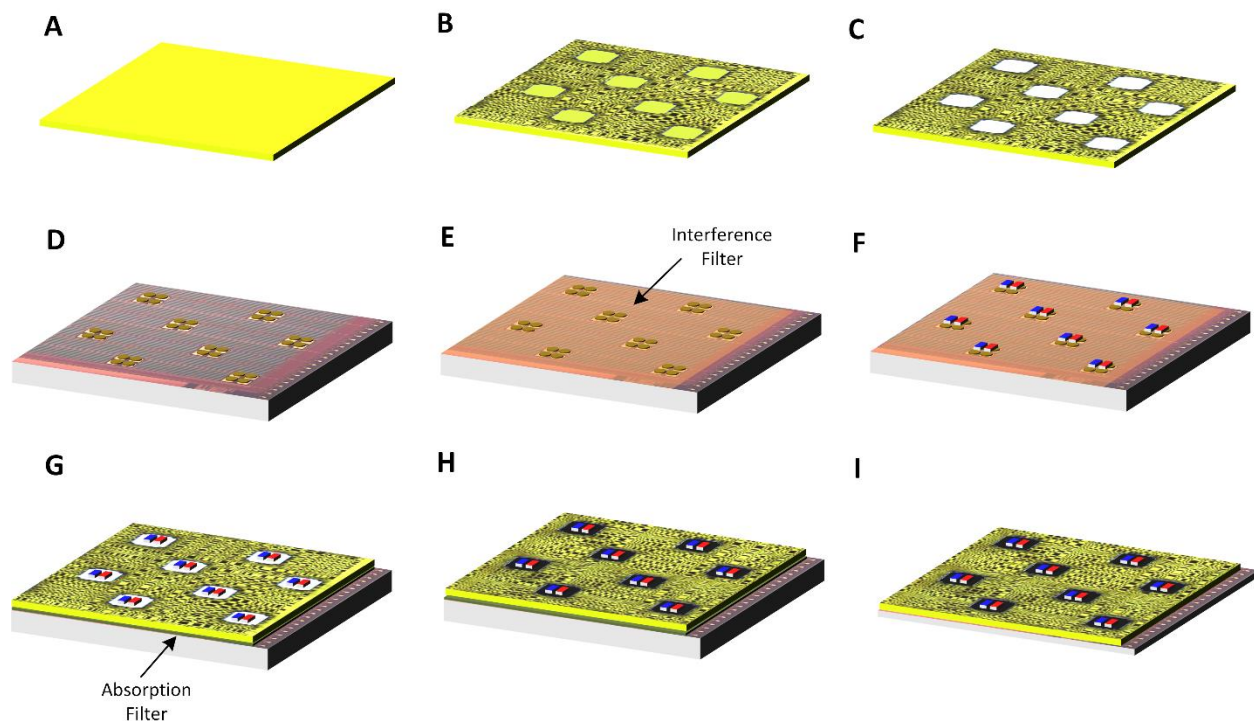


Fig. S5. Light sources, filters, and computational mask packaging. Flexible Packaging stack up with less than 250um thickness as well as a functioning die-thinned chip bending along a radius of curvature of 10mm. (A) Polyimide substrate layer (B) Computational mask with Cr patterning, (C) Excimer laser cut-outs, (D) CMOS chip with C4 bumps for μ LED contacts, (E) Thin-film dielectric interference filter coating, (F) Flip-chip bonding of μ LEDs, (G) Bonding of computational mask to the interference coated and μ LED bonded chip using custom absorption filter epoxy, (H) Black underfill epoxy of μ LEDs, and (I) CMOS substrate die-thinning. Note: The packaging flow is done in two parallel steps, the mask fabrication in steps (A)-(C) and the interference filter and μ LED bonding in steps (D)-(F). The two parallel flows are combined together in step (G) via an absorption filter adhesive bonding step.

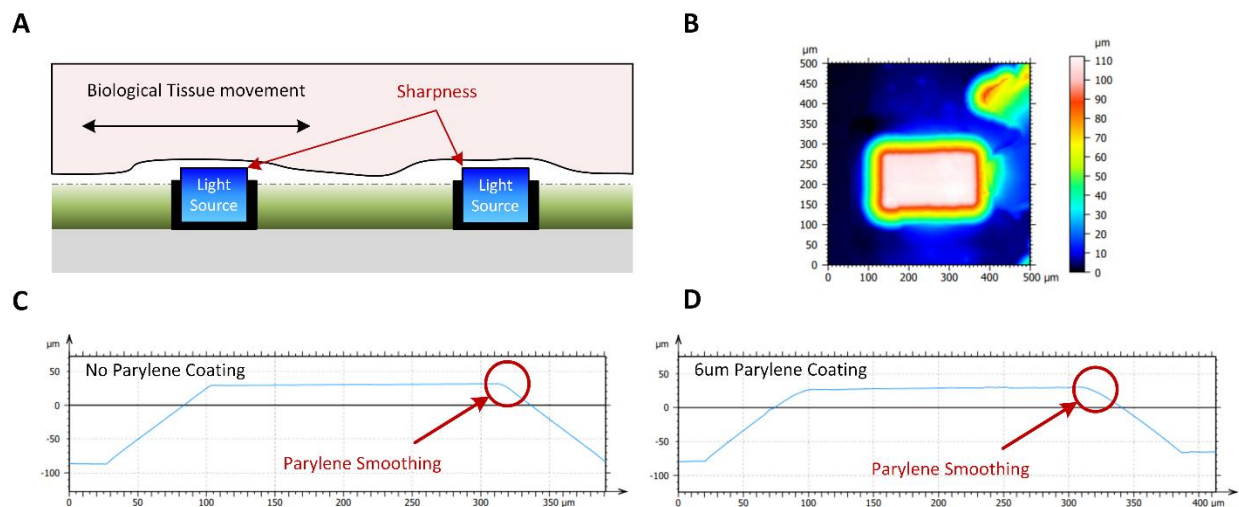


Fig. S6. LED sharpness. (A) Cross-section of the CMOS sensor with integrated light sources showing the potential for shearing due to biological tissue movement, (B) Topographical image captured using profilometer, (C) Comparison of smoothness of the μ LED before and after 6 μ m of parylene coating.

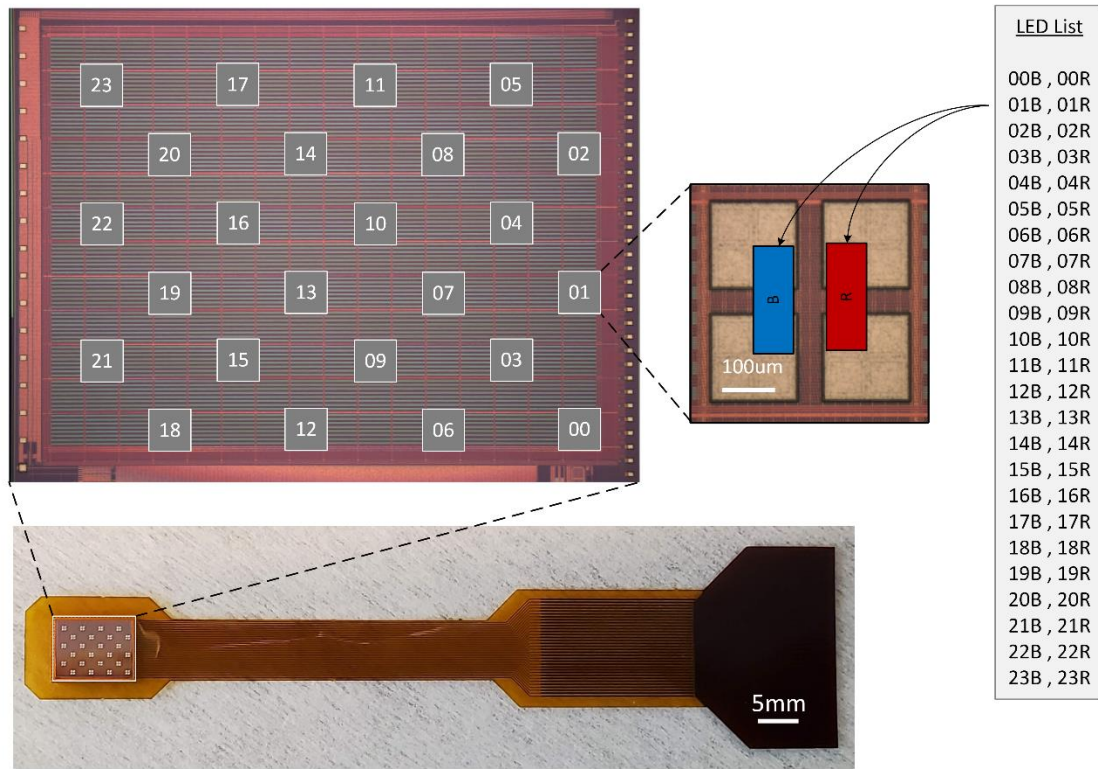


Fig. S7. LED map for orientation and localization in animal experiments. The LEDs are numbered in pairs from 00 until 23 with each pair containing a blue and red LED. In the electrical stimulation mouse experiment, the flexible shank electrodes provide biphasic current stimulation at LED pair location 10, and the blue recording LEDs are located at 07B, 08B, 09B, 10B, 13B, and 14B. In the optogenetic stimulation and optical recording mouse experiment, the red stimulation LED is located at 08R, and the quad of blue recording LEDs are located at 10B, 13B, 14B, and 16B. In the NHP experiment, all the blue LED locations are enabled for full FoV imaging. The dead LED locations correspond to 02B and 22B.

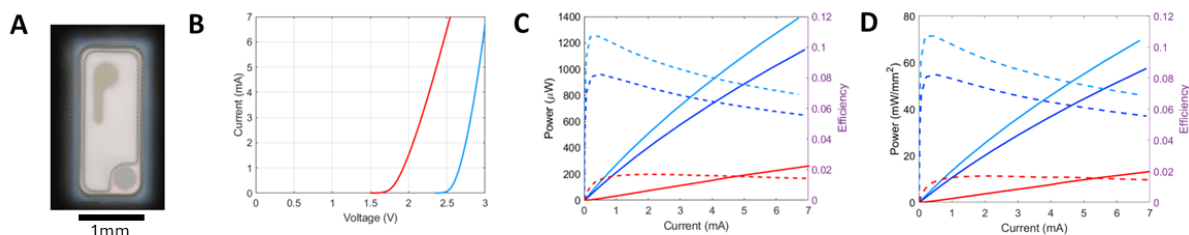


Fig. S8. LED electrical and optical characterization of LEDs. (A) Die photo of the LA UB08FP2 blue LED chip with a surface emission area of $0.2\text{mm} \times 0.1\text{mm}$. Anode contact is on the top pad and cathode contact is on the bottom pad (B) Current versus voltage sweep for blue and red LEDs using the drivers on the chip. (C) Measured output light power captured using ThorLabs PM100D photodiode module. The efficiency is calculated by taking the light power and normalizing it to the electrical power. (D) Output light power density taken by normalizing the output light power to the radiative surface area of the LED chip.

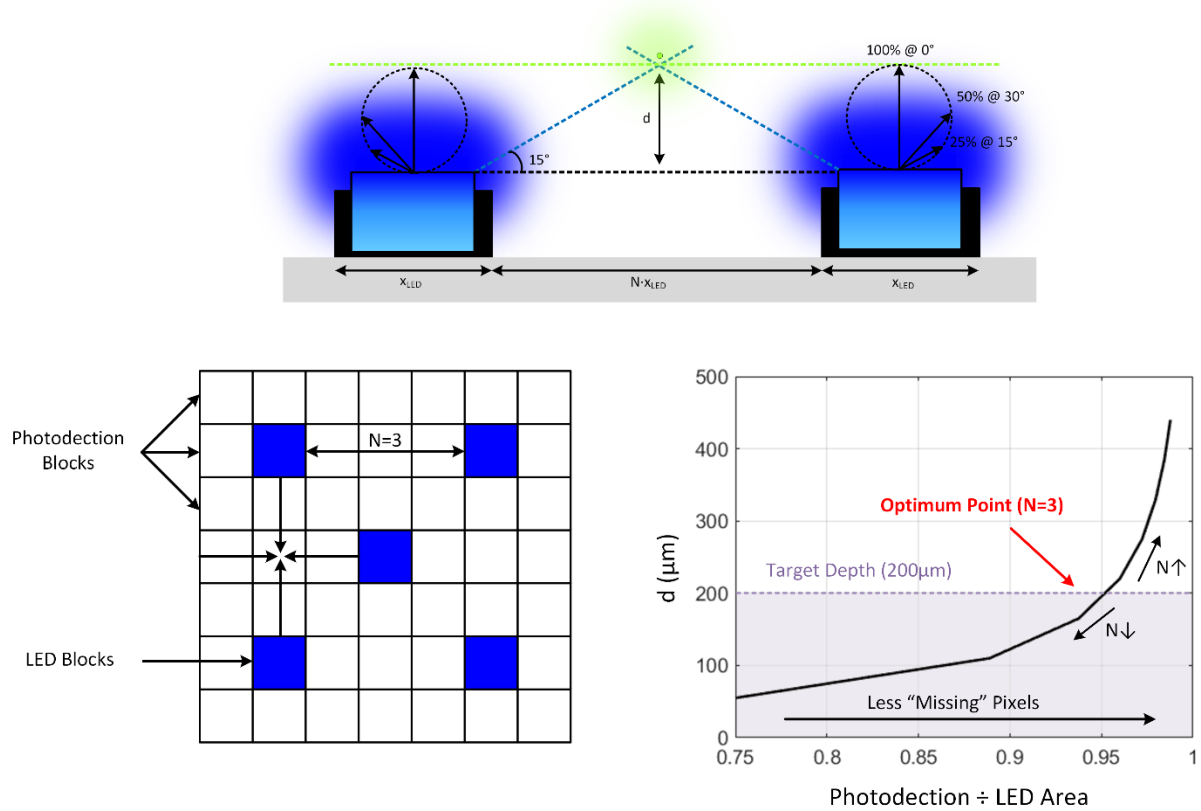


Fig. S9. Pitch of light sources. The illumination pattern is determined by the Lambertian radiation profile, tissue scattering, and the pitch between the μ LEDs. The Lambertian μ LED emission profile is means that emission angle that corresponds with 25% power output is 75° from normal incidence. The pitch between μ LEDs is optimized such that the target depth of 200 μm is covered within the FWHM of adjacent μ LEDs while allowing for the highest ratio of photodetectors to μ LED blocks. In this design, each photodetection block consists of 16x16 arrays of SPADs. Sweeping the number (N) of photodetection blocks between μ LEDs gives an optimum point of N=3 where there is FWHM overlap between adjacent μ LEDs yet still allows for 87.5% of the array to be covered by photodetectors. In this design we were limited by the μ LED sizes available commercially. Shallower depths of uniform illumination could have been achieved by going to smaller μ LED sizes while maintaining the same 87.5% photodetection-to-emission-area ratio.

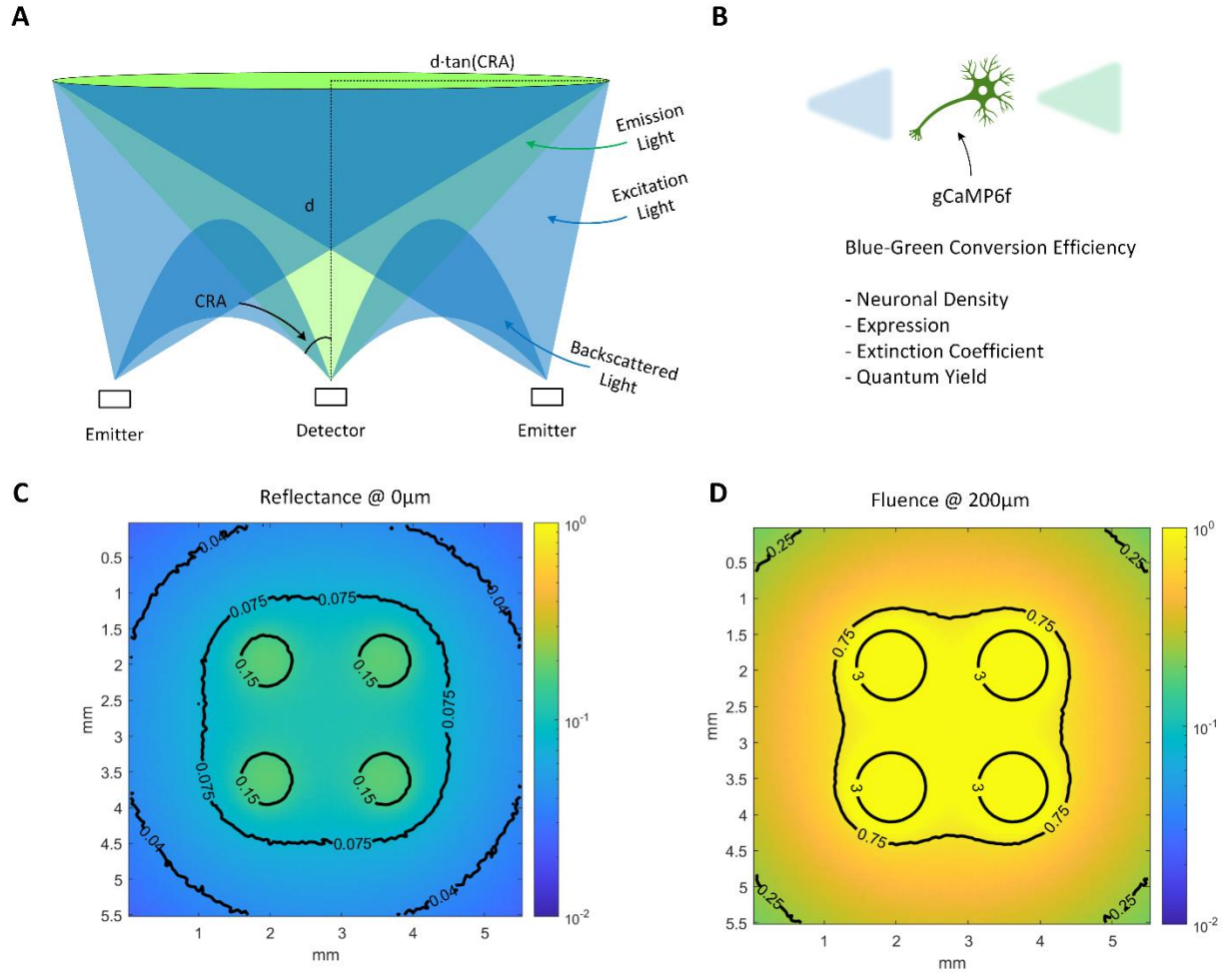


Fig. S10. Analysis of biophotonics of SCOPe. (A) Modeling of μLED excitation and backscattered light with respect to the source detector separation and geometry as well as the fluorescence emission and angular acceptance of the SPADs. (B) The fluorescence signal for a given volume is proportional to the intrinsic neuronal density of the tissue, the percentage of GCaMP6f expressing neurons, and the extinction coefficient and quantum yield of GCaMP6f. (C) The reflectance is the amount of light exiting the tissue at $z=0\mu\text{m}$ and is modeled using a Monte Carlo simulation using the μLED pitch and Lambertian source emission profile. (D) The fluence is the amount of light at the $z=200\mu\text{m}$ plane normalized to the μLED light source power. Due to the effects of scattering, there is a focusing of power density to the superficial layers of the tissue resulting in lower required μLED light emission.

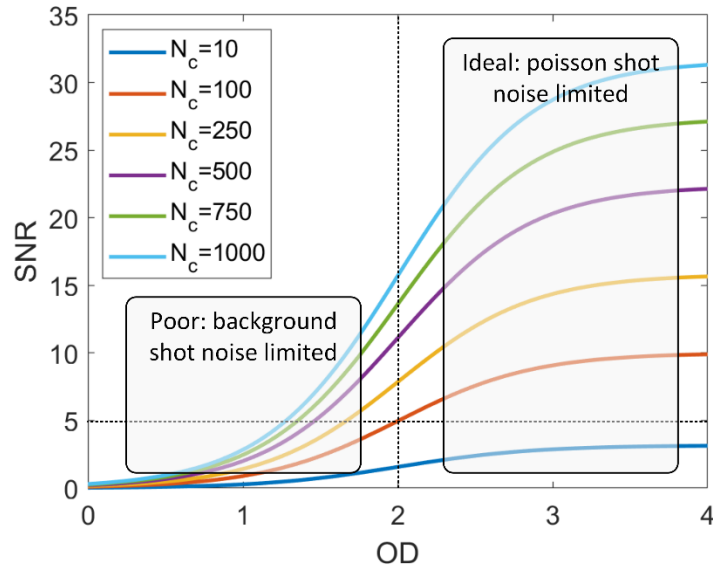


Fig. S11. Filtering equations with SNR. The SNR curves corresponding with the equations in **Section S9** for various sensor counts per frame and filtering optical densities. As expected, the SNR curves increase with SPAD sensor counts and with OD filtering improvements. For $OD < 2$, the SNR is limited by background shot-noise, and for $OD > 2$, the SNR is limited by Poisson shot noise with limited improvements due to additional filtering. For example, to achieve an SNR of 5 while restricting the dynamic range to only 40 dB, which is less than 10% of full-scale and corresponds to the excitation light levels employed in these experiments, an OD of 2 is required.

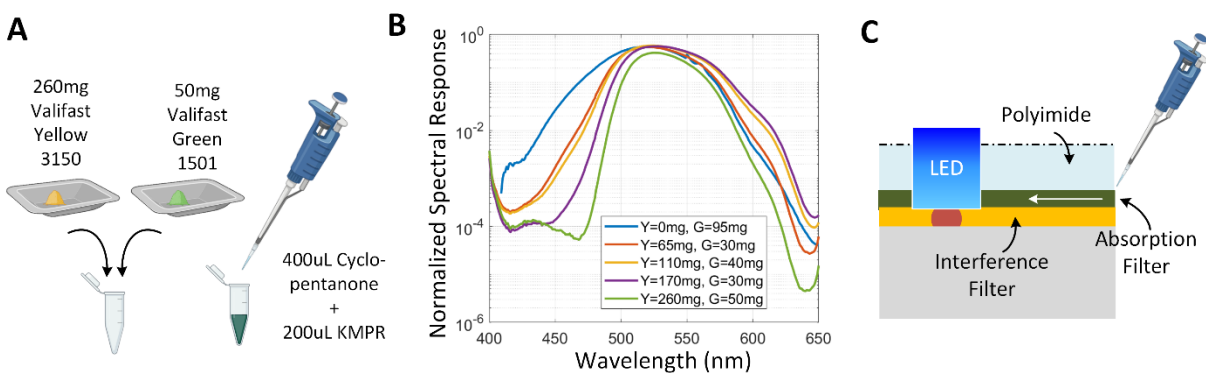


Fig. S12. Optimization of absorption filter. (A) The absorption filter is synthesized using a combination of 260mg Valifast Yellow 3150, 50mg Valifast Green 1501, 400 μ L Cyclopentanone, and 200 μ L KMPR, (B) Spectral transmission plots for custom absorption filters using various quantities of Valifast Yellow and Green dyes, and (C) The polyimide spacer used for the computational mask also serves as a top substrate for injection of the absorption filter using capillary force for a final thickness of $\sim 10\text{-}20\mu\text{m}$.

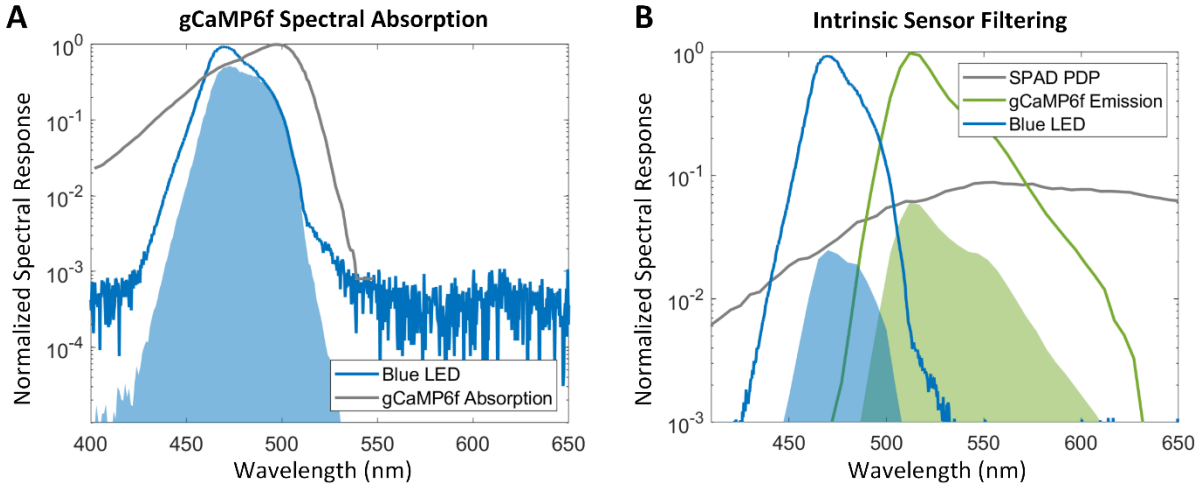


Fig. S13. Spectral sensitivity. (A) The spectral overlap of the blue excitation LED with the excitation filter and the GCaMP6f absorption spectrum. Due to the peak mismatch and the spectral bandwidth of the light source, the resulting spectral overlap is 60%. (B) The sensor pixel has a photon detection probability that varies across wavelength with slightly greater sensitivity in the green and red wavelengths. Integrating the blue excitation LED spectrum gives an integrated photon detection probability of 3.1% and the GCaMP6f emission gives an integrated photon detection probability of 6.75%. Taking the ratio of these two integrated PDP values gives the sensor an intrinsic filtering of 2.2:1.

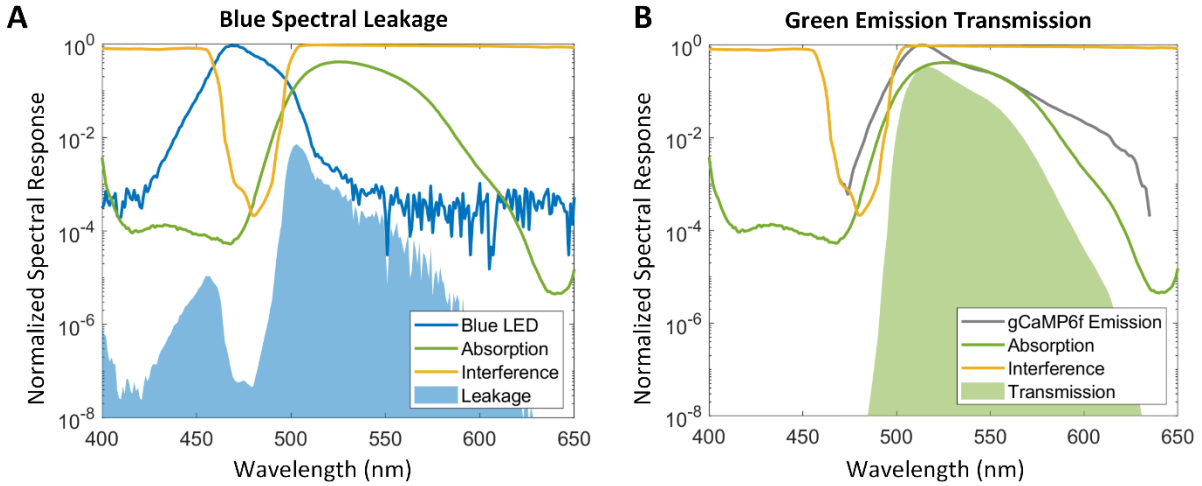


Fig. S14. Filter characterization. (A) The total spectral leakage of the blue LED through the excitation and emission filter stack. The interference provides steep transition between the stopband and passband while the absorption filter provides wide stopband rejection across the blue LED spectrum. Leakage is calculated by multiplying the spectral responses together and integrating across the entire frequency domain resulting in a leakage of 0.0037 and contributing to the background in sensor captures. (B) The total spectral transmission of the GCaMP6f fluorescence through the emission filter stack is 0.2677. The total OD filtering is the ratio of these two values which results in $OD > 1.9$, or 72:1 rejection of light source photons in favor of emission photons.

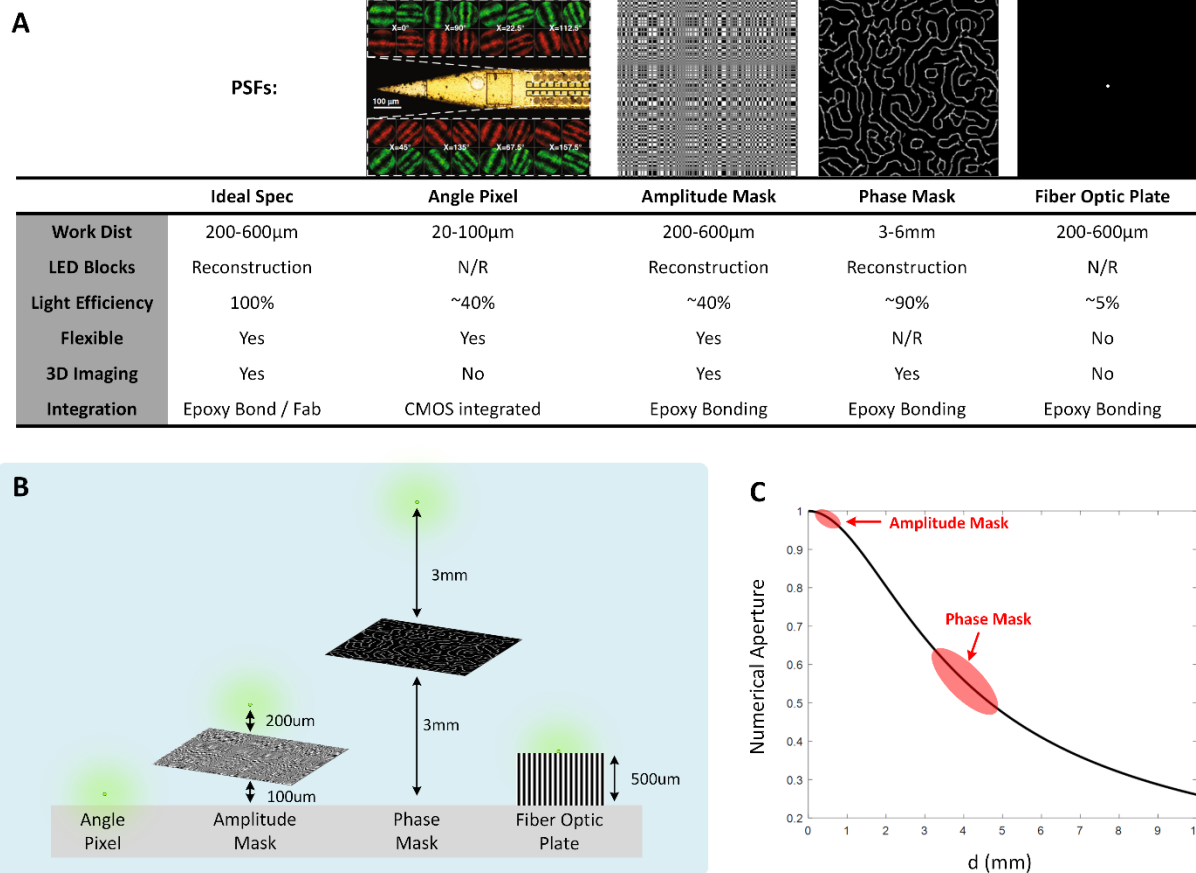


Fig. S15. Comparison of computational mask designs. (A) We evaluated various types of computational imaging masks on the basis of working distance, reconstruction with LED blocks, light collection efficiency, flexibility, 3D capabilities, and ease of integration. (B) Comparison of working distances of the angle pixel⁵⁶, amplitude mask^{1,57}, phase mask³⁰, and fiber optic plate. (C) Numerical aperture, comparing amplitude and phase masks.

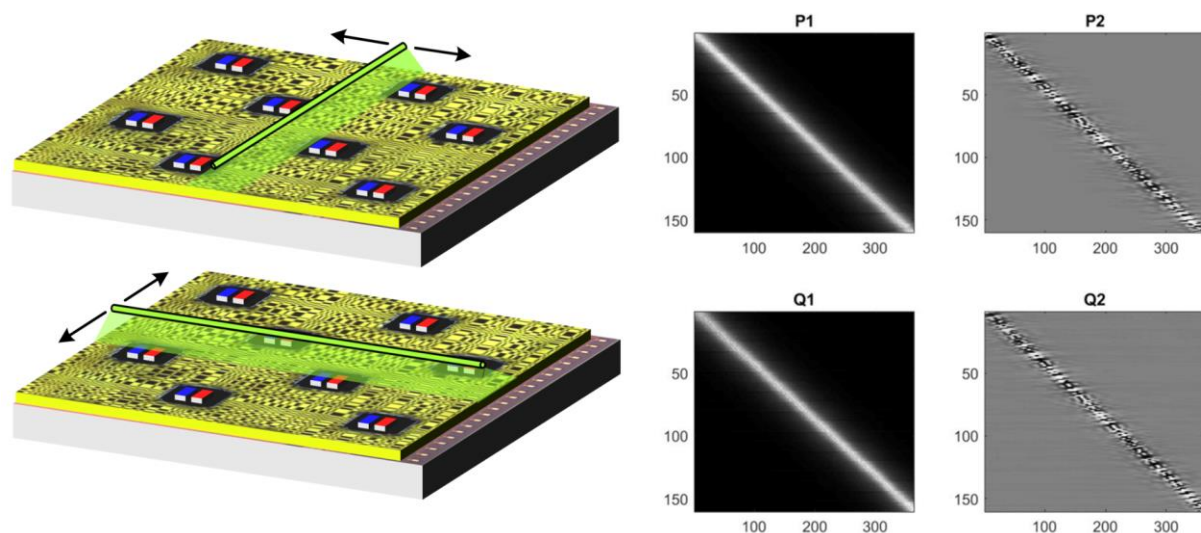


Fig. S16. Computational imaging flow – calibration. Texas Two-Step algorithm allows for reduction in calibration time and data storage based off of separable mask design, calibration setup showing the sweeping of a line slit in row / col directions, the P_1 , P_2 , Q_1 , and Q_2 calibration matrices where the P_1 , P_2 matrices encode the intensity information, and the Q_1 , Q_2 matrices encode the mask modulation information.

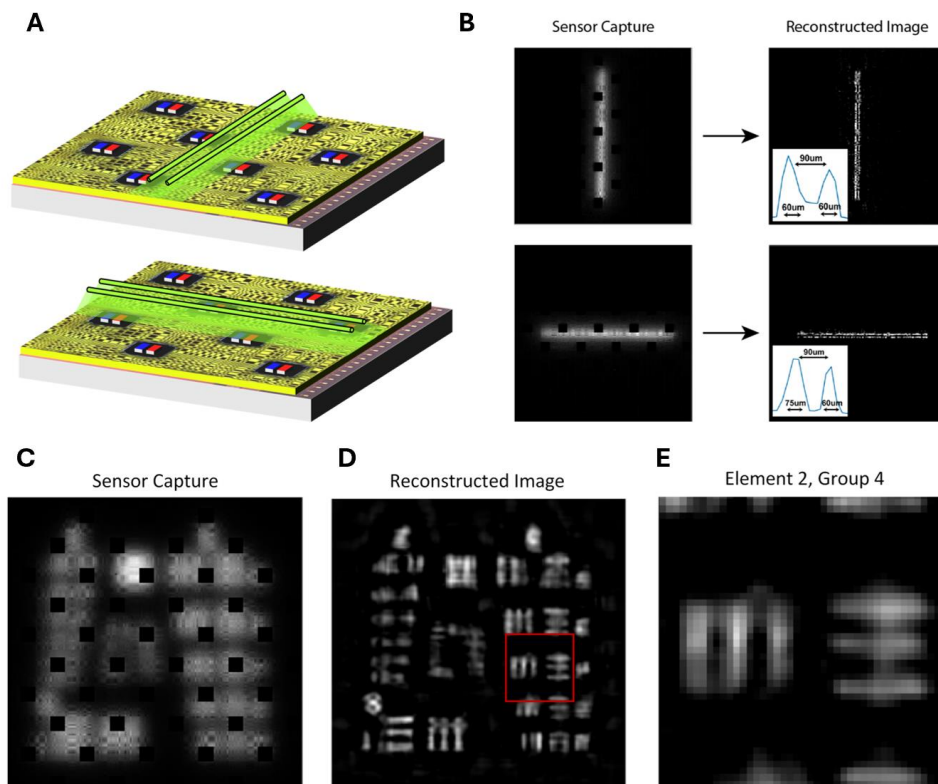


Fig. S17. Computational imaging flow – reconstruction. Overall computational imaging flow including calibration, calculation of calibration matrices, raw image capture and computational reconstruction using an inverse optimization algorithm (A) Double line slit reconstruction setup for demonstrating resolution in the row and column dimensions, (B) Double line slit reconstruction at 200 μm depth showing the resolution of the sensor to be better than 60 μm , (C) The raw sensor capture for a USAF resolution target showing the missing information due to LED blocks, (D) Reconstructed image capture of the USAF resolution target at 200 μm depth (E) Magnified inset of element 2, group 4 of the resolution target showing better than 60 μm resolution.

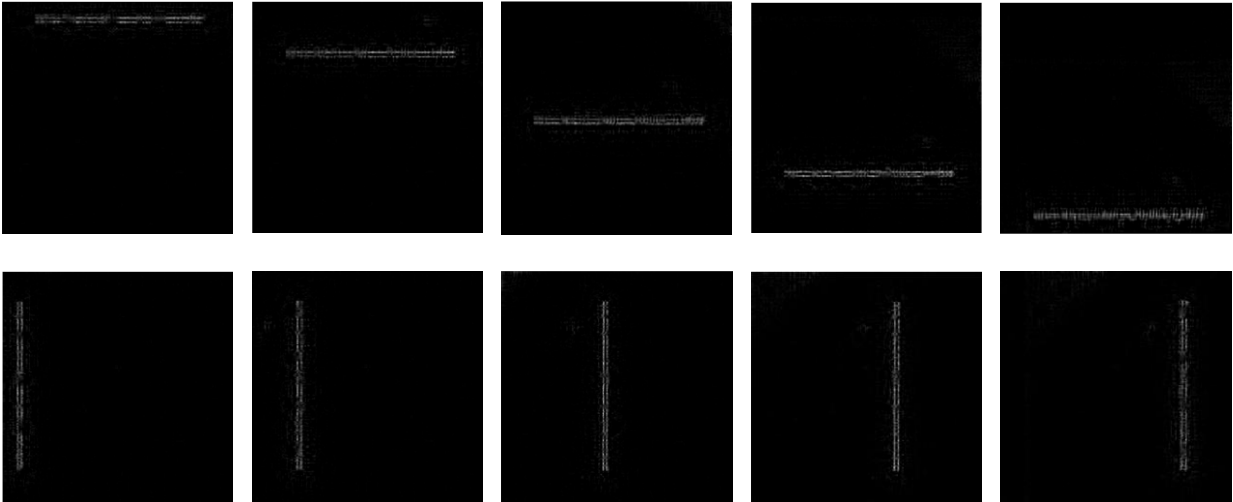


Fig. S18. Reconstruction variation across the image sensor. Reconstruction measurements at 100 μm depth showing the resolution of the sensor to be better than 60 μm across the sensor.

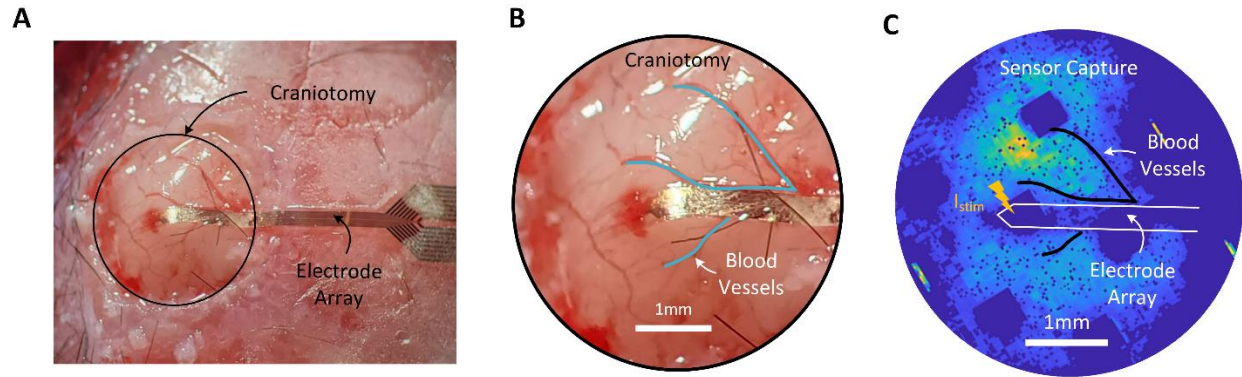


Fig. S19. Surgical preparation for electrical stimulation in mouse experiment. (A) The craniotomy preparation including a $6 \times 6 \text{ mm}^2$ cranial window over the SSC and VC of the left hemisphere of the anesthetized mouse. The flexible shanks are laminated across the cortex and implanted deep into the SC down to Layer 5 for application of electrical current stimulation with varying amplitudes. (B) White light microscope image of the cranial window showing the insertion location of the flexible shanks relative to major blood vessels. (C) The SCOPe image capture is aligned according to these experimental and physiological features to locate the stimulus location. Images are captured at 40 Hz and the full image is cropped to only pixel locations that are in contact with the neural tissue in the cranial window. Blood vessels $>14 \mu\text{m}$ diameter account for $<1.4\%$ of the vessels in the mouse brain⁵⁸ (DOI: 10.1016/j.jneumeth.2016.06.012). As evident from the Monte Carlo blood vessel simulations, we expect only to see the largest ($>50 \mu\text{m}$) and superficial blood vessels. Over the $2.1 \times 3 \text{ mm}$ FoV, we cross-validate three of the largest blood vessels with white light camera photos.

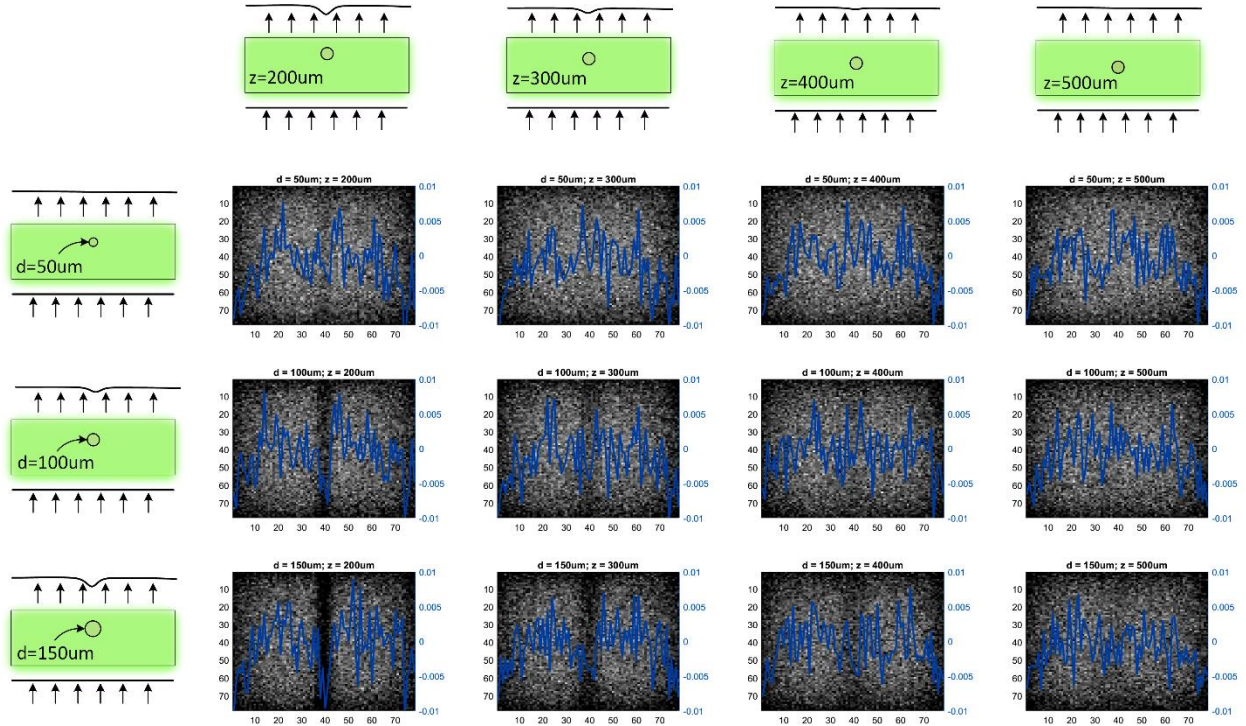


Fig. S20. Blood vessel imaging. Monte Carlo setup of a dense fluorescence scene and the occlusion of a single blood vessel of diameters of 50, 100, and 150 μm (along the row axis) and z-depths of 200, 300, 400, and 500 μm (along the column axis). A 3×4 matrix of the transmittance at the tissue-air interface of the fluorescent scene. The blue line overlay shows the cross-sectional counts at the middle row. To image a contrast of $\sim 1\%$ with an $\text{SNR} > 3$ necessitates the detection of $N > \left(\frac{3}{0.01}\right)^2 = 90,000$ photons.

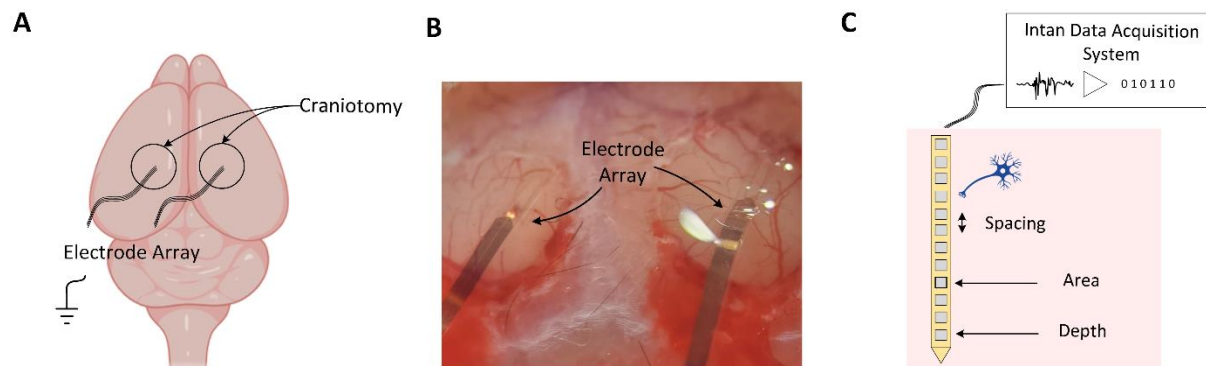


Fig. S21. Surgical preparation for optogenetic stimulation in mouse experiment. (A) The craniotomy preparation including a 6×6 mm² cranial window over the SSC and VC of the left hemisphere of the anesthetized mouse. The flexible shanks are laminated across the cortex and implanted deep into the SSC down to Layer 5 for application of electrical current stimulation with varying amplitudes. (B) White light microscope image of the cranial window showing the insertion location of the flexible shanks relative to major blood vessels. (C) The sensor image capture is aligned according to these experimental and physiological features to locate the stimulus location. Images are captured at 40 Hz and the full image is cropped to only pixel locations that are in contact with the neural tissue in the cranial window.

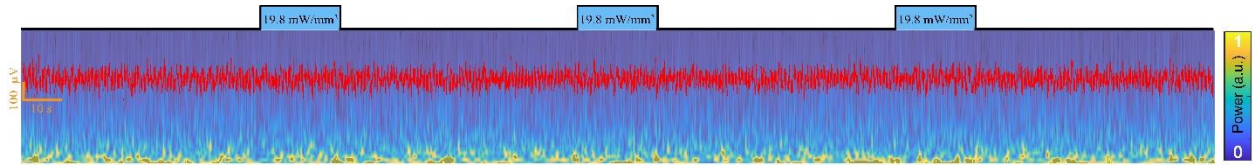


Fig. S22. Control experiments for neural activity modulation *in vivo* in wild type mice. LED activation over the implanted electrode array does not show any LFP modulation or artifact generation before and after stimulation.

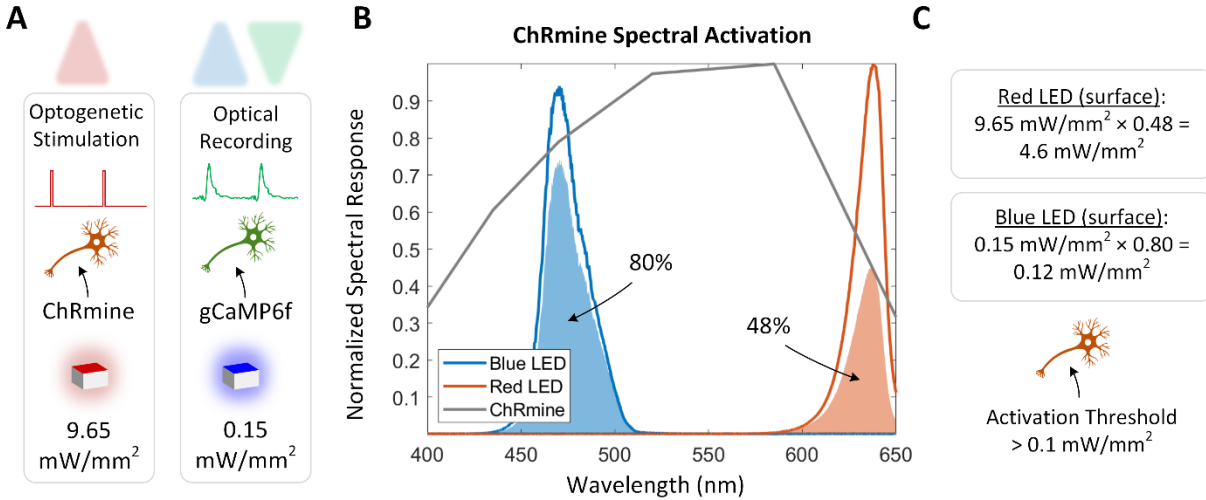


Fig. S23. Spectral overlap of LEDs and ChRmine opsin. (A) In the optogenetic stimulation, optical recording in vivo mouse experiment, the operating point of the red LED for stimulation was set at 9.65mW/mm² and blue LED for recording was set at 0.15mW/mm² (**Table S5**). (B) The spectral overlap of the blue excitation LED with the excitation filter and the ChRmine activation spectrum is 80%. The spectral overlap of the blue excitation LED with the excitation filter and the ChRmine activation spectrum is 48%. (C) This corresponds to an emitted red stimulation light power density of 4.6mW/mm² and blue recording light power density of 0.12mW/mm². The activation threshold for ChRmine is 0.1 mW/mm²³¹.

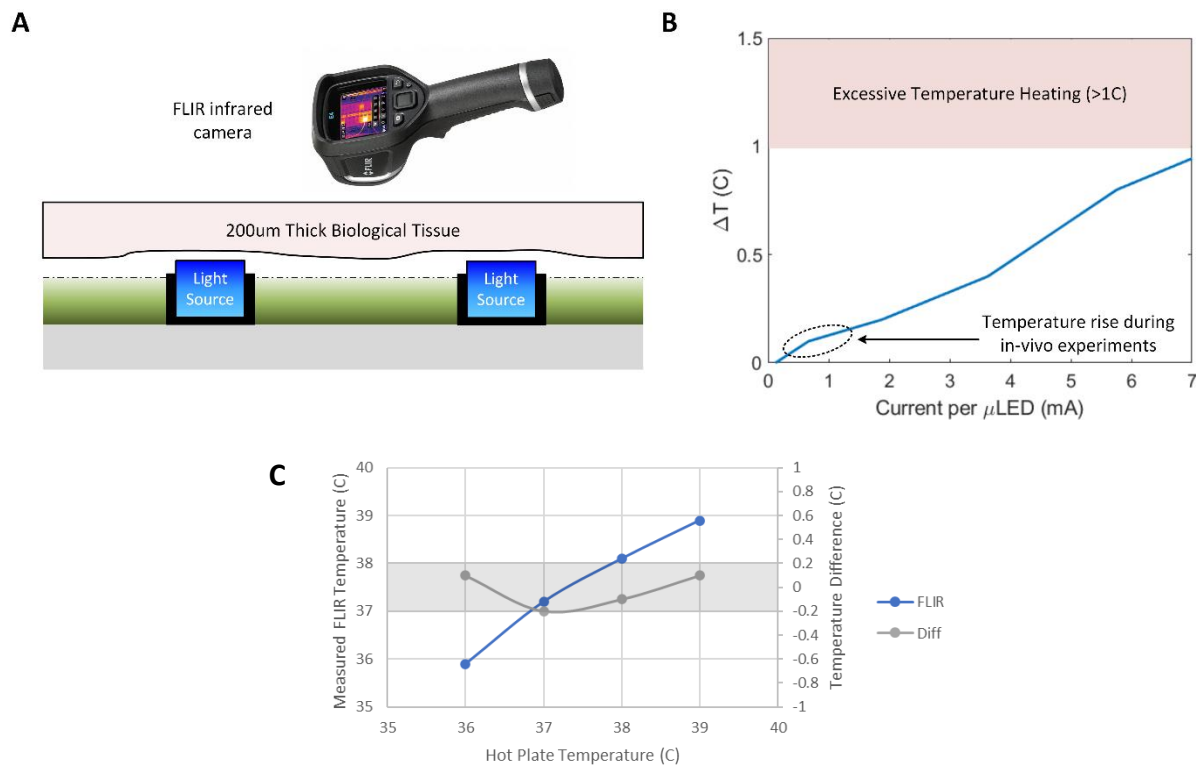


Fig. S24. Tissue heating. (A) Cross-section of the CMOS sensor with integrated light sources showing the heating of a 200 μ m thick tissue sample due to LED light generation and power dissipation and measured using an FLIR infrared camera. (B) Plot with the rise in temperature versus the current consumed in each μ LED; the temperature rise during in-vivo experiments in circled showing that the heating is negligible compared with the safety limits. (C) Plot showing the calibration of the FLIR camera used in the experiment across biologically relevant temperatures (36°C – 39°C) on a temperature-controlled surface. Accuracy is better than 0.2 °C.

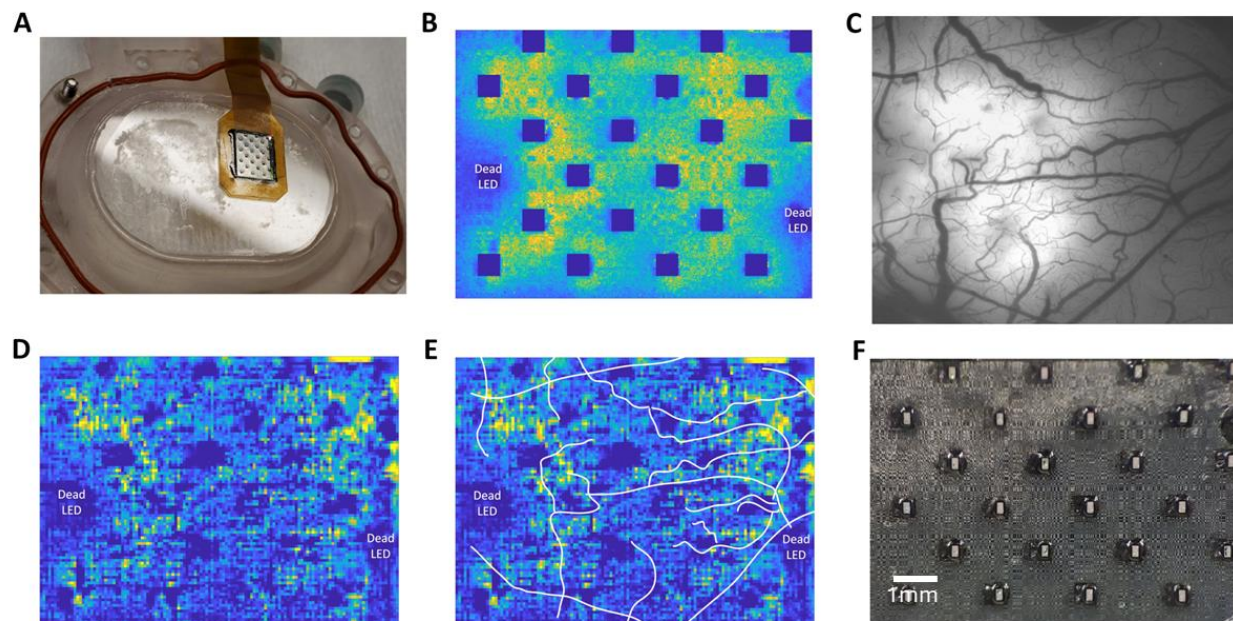


Fig. S25. NHP reconstruction. The device is packaged with all the necessary (24) blue μ LEDs, excitation and emission filters, and biocompatible parylene coating. The targeted location on the brain port is for the PMd and M1 brain regions. (A) Camera photo taken of the device prior to implantation. The SCOPe is epoxy bonded on top of the cranial window and sterilized. (B) The non-binned spatial capture (192×256 pixels) averaged over the entire 50 seconds of a reaching recording. There are 22/24 functional μ LEDs on the chip allowing to take advantage of the entire sensor for imaging. (C) Ground truth image of GCaMP8m fluorescence expression in the cortical region under the sensor showing the blood vasculature. (D) Sensor reconstruction image refocused to a calibration depth of $200\mu\text{m}$. (E) The refocused image with the blood vasculature overlaid in white lines. (F) Microscope capture of the SCOPe sensor post-experiment showing fully intact device capable of repeat experiment after another sterilization process. There is some biological residue in the top left corner of the chip which also will account for reduced performance in the image reconstruction process.

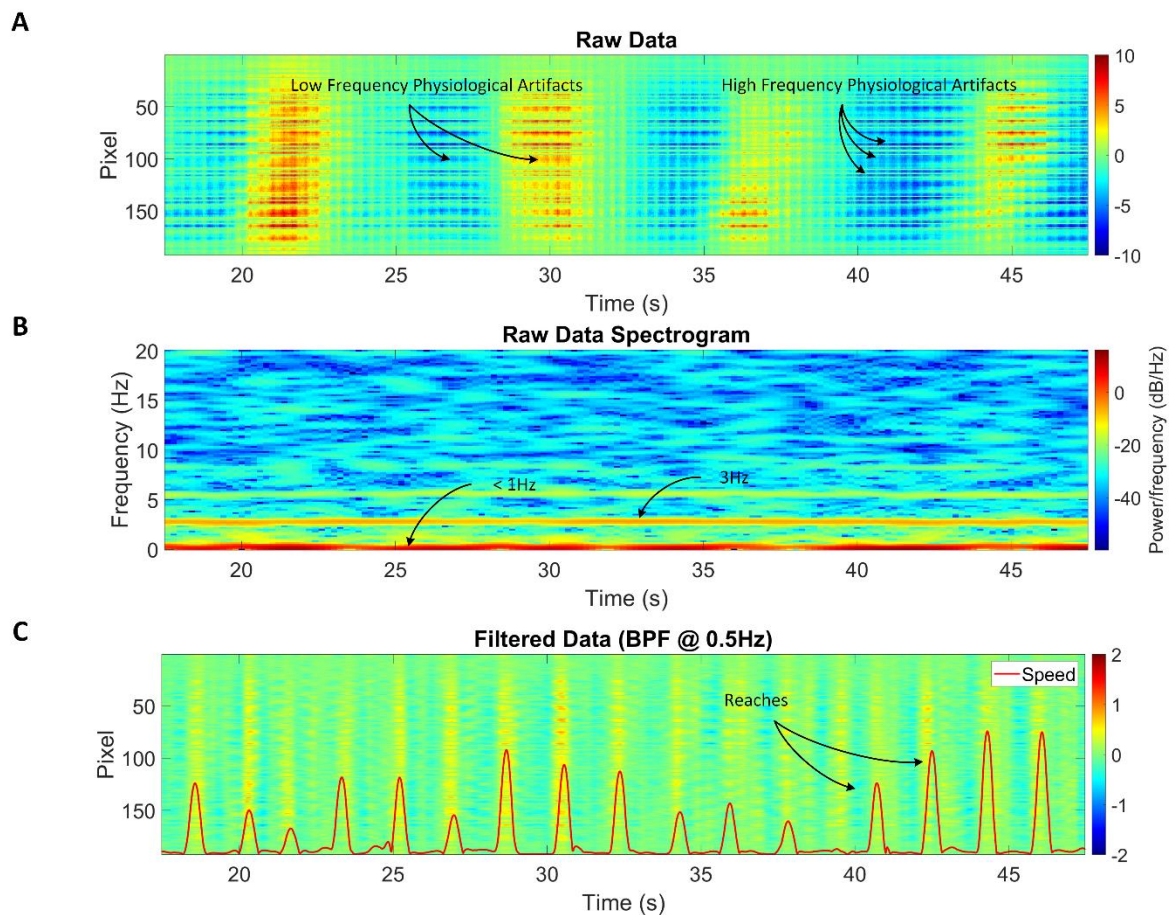


Fig. S26. Spectrogram. (A) Example of the space-time matrix of the raw data captured by the sensor containing physiological artifacts. (B) Low frequency 0.1-Hz components are related to blood oxygenation and high frequency 3Hz components are related to heartbeat. Blood oxygenation is expected to have minimal contribution to contamination of the fluorescence signal due to the small differences in absorption between oxyhemoglobin and deoxyhemoglobin in the green wavelengths⁴². On the other hand, increases in blood volume can cause a slight decrease in both the blue excitation and green emission light. One way to correct blood volume is to employ a multi-wavelength strategy by measuring the ratio of green light reflectance to blue light reflectance and subtracting from the raw data to reduce the blood volume hemodynamic response. However, in practice, the activity dependent GCaMP signals are large enough to overcome the

contribution of blood volume to fluorescence signals ($<10\%$)^{36,40}. The strongest mixing of GCaMP neural signals with hemodynamics are in the 0-0.4 Hz frequency band⁴³. In cases without dual-wavelength hemodynamic correction, the GCaMP signals can be extracted by employing temporal high-pass digital filters with 3dB cutoffs at either 0.1 Hz^{38,44} or 0.5 Hz⁴⁵⁻⁴⁷ since most of the mesoscale functional imaging power is located in the delta band (0.1-4Hz). (C) Space-time matrix of the temporally bandpass filtered data showing neural activations that are timed synchronously with the reaching tasks.

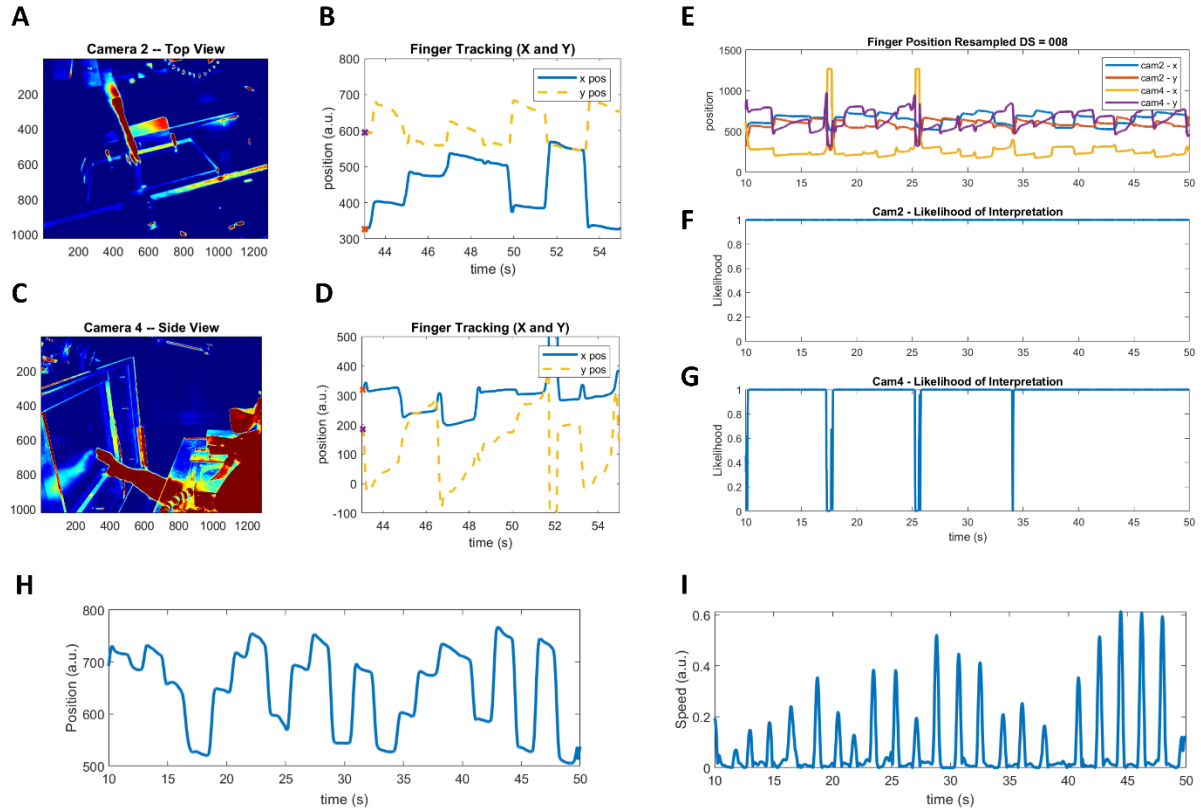


Fig. S27. DLC finger tracking. The finger tracking position is provided by synchronizing and mapping in the x and y direction the position of the finger in each of the successive frames, and it is used to identify the onset and completion of reaching tasks and map to the sensor data capture. (A) Camera two top view infrared camera for finger tracking in left-right and forward-backward dimensions. (B) Finger tracking positions as output from the DLC pipeline with the left-right positions (blue line) and the forward-backward positions (yellow dotted line). (C) Camera four side view infrared camera for finger tracking in the up-down dimension and forward-backward dimensions. (D) Finger tracking positions with the up-down positions (blue line) and the forward-backward dimension (yellow dotted line). (E) The finger position given for 40 seconds of experimental data for camera 2, x and y positions, and camera 4, x and y positions. (F) The DLC likelihood of interpretation for camera 2; a likelihood of 1 represents a correct positional estimation,

and a likelihood of 0 represents an incorrect positional estimation. Incorrect positional estimations occur due to moments when the finger position falls outside the field of view of the camera. (G) The DLC likelihood of interpretation for camera 4. (H) The estimated finger position given for 40 seconds of experimental data for camera 2, x position and temporally smoothed in time by 10 samples. (I) The corresponding speed calculated from the absolute value of the first derivative of position and temporally smoothed in time by 10 samples.

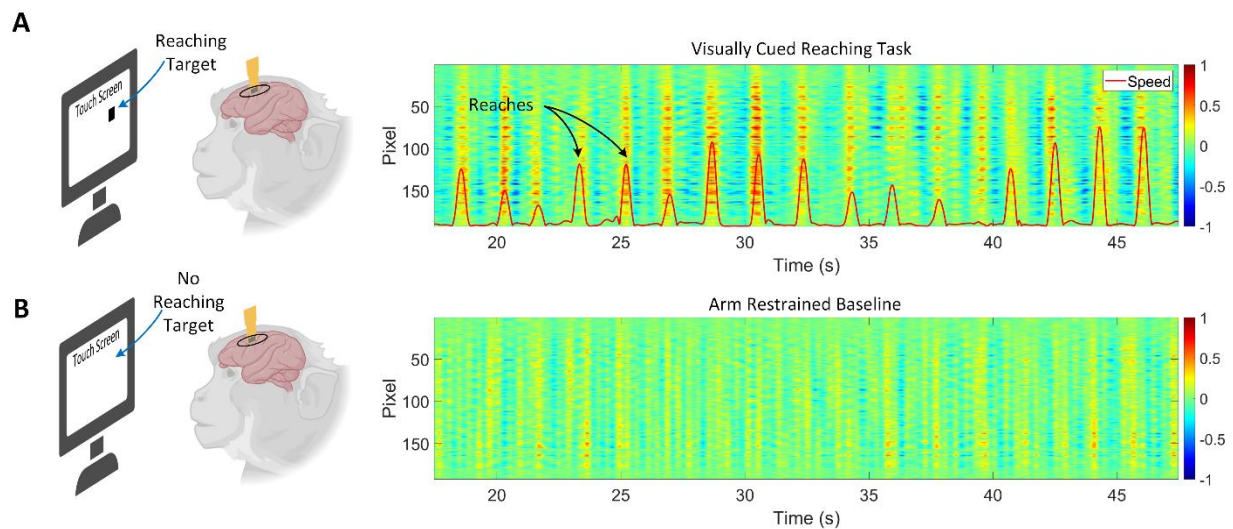


Fig. S28. Reaching versus arm-restrained baseline activity. (A) With no arm restraints, activity is synchronized to reaching events and shows good correlation between neural activity and finger speed. (B) The same activations in neural response do not exist in the arm-restrained baseline experiment.

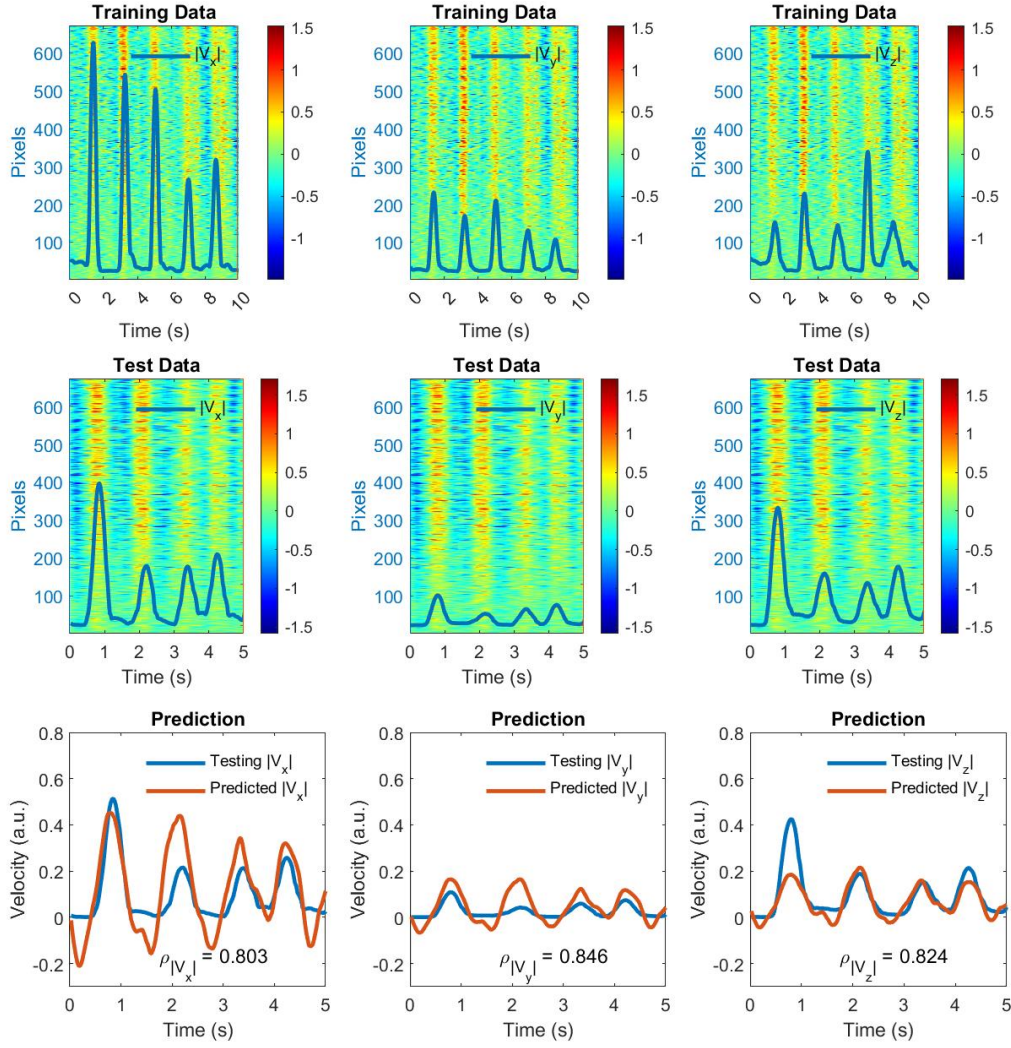


Fig. S29. Directional speed feature decoder. (Left, top) ten second training dataset with (blue trace) monkey directional reaching speed, $|V_x|$, overlapped with SCOPE spatiotemporal matrix. (Left, middle) five second testing dataset with (blue trace) monkey directional reaching speed, $|V_x|$, overlapped with SCOPE spatiotemporal matrix. (Left, bottom) Overlapping of (red trace) model directional speed prediction, $|V_x|$, versus (blue trace) ground truth reaching speed, $|V_x|$, with corresponding correlation coefficient ($\rho_{|V_x|} = 0.803$). (Middle, top through bottom) Same analysis done with (blue trace) monkey directional reaching speed, $|V_y|$, with corresponding correlation coefficient ($\rho_{|V_y|} = 0.846$). (Right, top through bottom) Same analysis done with (blue trace) monkey directional reaching speed, $|V_z|$, with corresponding correlation coefficient ($\rho_{|V_z|} = 0.824$).

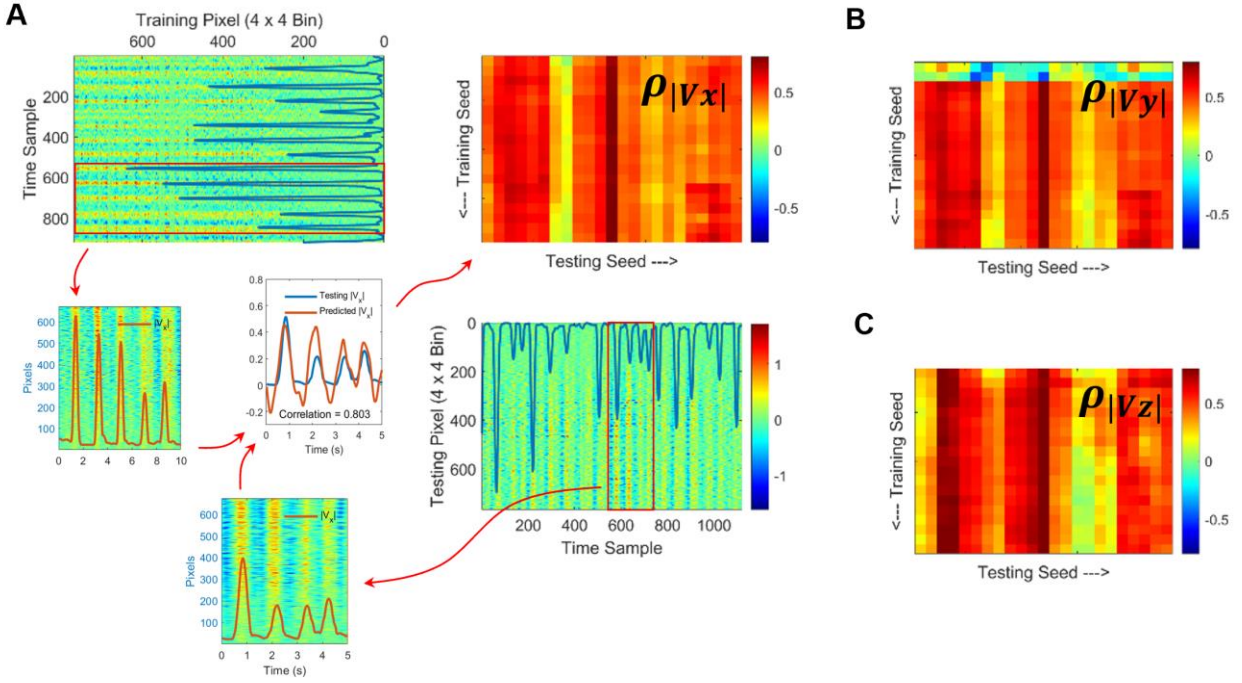


Fig. S30. Speed feature decoder correlation matrix. To demonstrate the performance of the directional speed decoder across the entire dataset (fifty second recording), we swept the training (ten second window) and testing (five second window) data subsets relative to each other and recorded the results in a correlation matrix. With all the monkey datasets, the first fifteen seconds are removed since there are transient artifacts due to the turning on of the LED light sources. The remaining 1400 samples (35 seconds * 40 samples/s) are plotted in a 19×23 correlation matrix by sweeping the training (10 second, 400 sample) and testing (5 second, 200 sample) windows back and forth in 1.25 second (50 sample) increments. (A) The directional speed ($|V_x|$) feature decoder matrix with (blue trace) monkey directional reaching speed, $|V_x|$, overlapped with SCOPE spatiotemporal matrix training seed on the rows and testing seed on the columns. Speed (blue line, arbitrary units) at each time sample overlaid with the training and testing space-time datasets. The decoder correlation matrix with respect to speed gives a time average correlation of $\rho_{|V_x|} = 0.472$. (B) Speed ($|V_y|$) Feature Decoder matrix with a time average correlation of $\rho_{|V_y|} = 0.436$. (C)

Speed ($|V_Z|$) Feature Decoder matrix with a time average correlation of $\rho_{|V_Z|} = 0.484$. The same analysis was performed for the signed velocities showing lower correlations of $\rho_{V_x} = 0.152$, $\rho_{V_y} = 0.063$, $\rho_{V_z} = 0.376$.

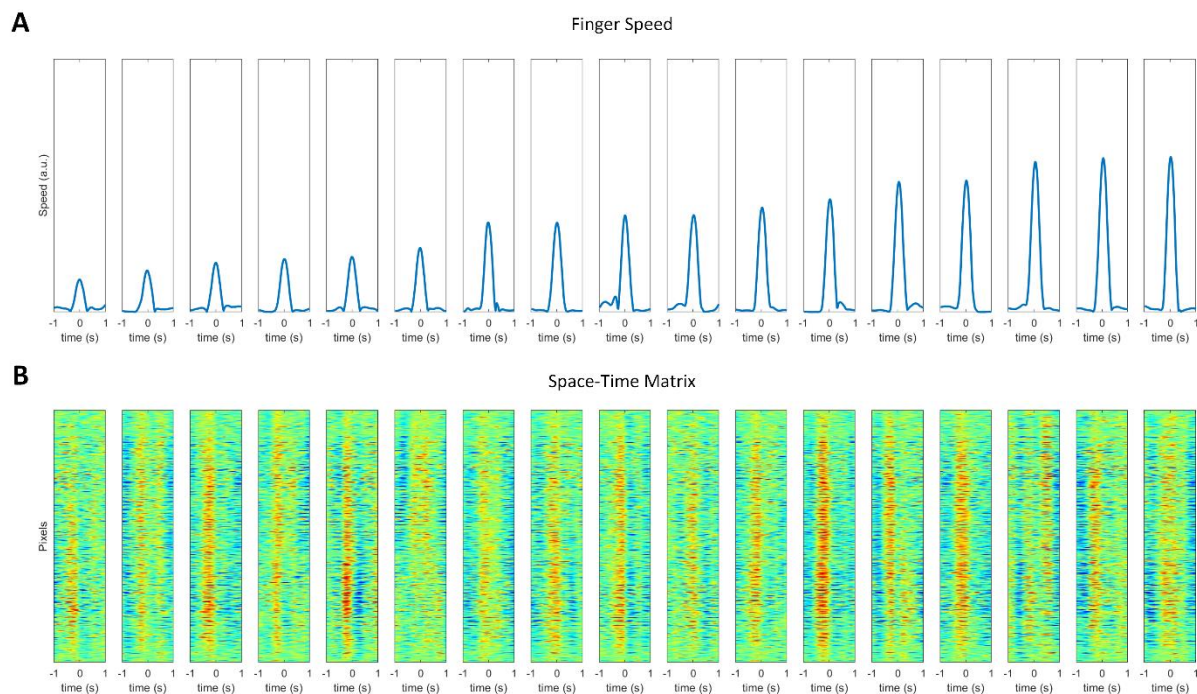


Fig. S31. Individual reaching events from a single recording. (A) The 17 individual reaching events are extracted and synchronized by indexing the time sample of the peak speed of the DLC tracked finger. (B) A window of ± 40 samples from the seed time sample are used to show the neural activity within ± 1 second of onset and termination of reaching movement.

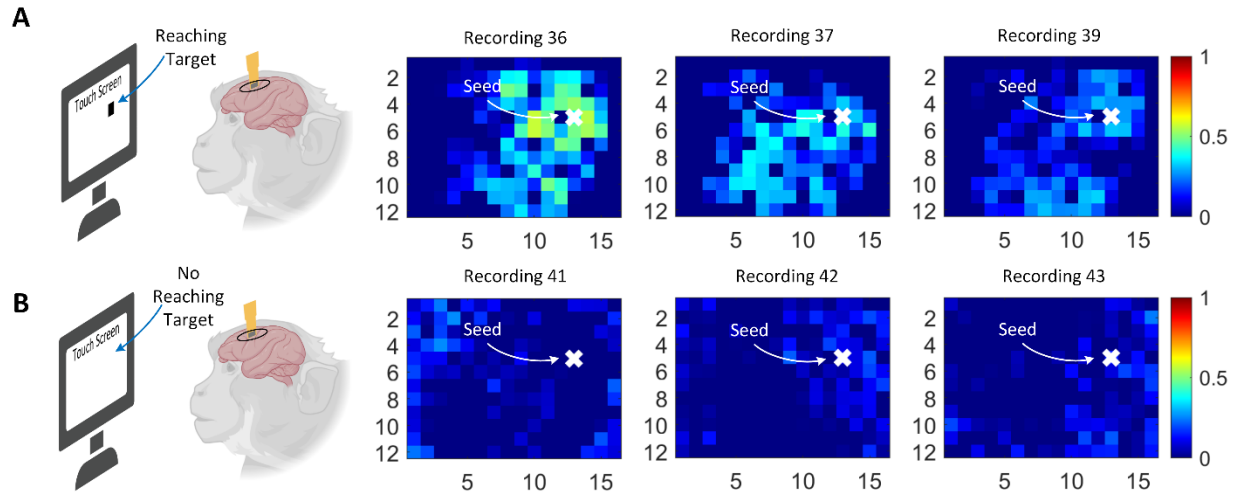


Fig. S32. Spatiotemporal correlations. The 2D spatial image shows the correlations at a time lag of 250ms (10 samples) between the seed pixel and the rest of the pixels in the array. The seed pixel is denoted with a white ‘X’ located in the top right of the array corresponding to the PMd brain region on the image sensor. The bottom-left quadrant corresponds to the M1 region and emphasizes the correlation at a neural lag of 250ms. (A) Recordings 36, 37, and 39 correspond with reaching experiments. The positive correlations in bottom-left quadrant, representing the M1 region, demonstrate that the neural activations travel from PMd to M1 with a time lag of 250ms. (B) Recordings 41, 42, and 43 correspond with baseline experiments with no visually cued reaches.

	Ref ⁵⁹	Ref ⁶⁰	Ref ⁶¹	Ref ⁶²	Ref ⁶³	Ref ³⁸	Ref ⁶⁴	Ref ³⁰	This Work
Name	Original	Wireless	MiniScope	CM ²	Mini-3D	mScope	2P MiniScope	FlatScope	SCOPE
Type	1P	1P	1P	Lensless	Lensless	1P	2P	Lensless	Lensless
Weight (G)	1.9	1.8	4	19	2.5	4	4.2	3	0.107
Size (mm³)[†]	10×10×15	20×10×10	25×15×15	29×29×30	10×10×17	25×15×15	16×9×30	8×8×8	6.4×7.8×0.15
FoV (mm³)[†]	0.6×0.8×0.2	0.6×0.8×0.2	0.8×0.8×0.4	8×7×2.5	0.9×0.7×0.39	8×10×0.2	0.42×0.42×0.18	4×4×0.5	5.1×6.8×0.5
Displacement	16,000	28,000	22,000	180	7,000	350	140,000	64	0.5
Res (μm)	2.5	4	6	7	2.76	39.37	1.13	10	60
Power (mW)[‡]	160	350	363	381	900	226	N/R	2,500	10 [§]
Bidirectional	No	No	No	No	No	No	No	No	Yes

Table S1. Comparison table with state-of-the-art miniscopes. Table compares the type (1P, Lensless, 2P), weight, size (mm³), FoV(mm³), displacement factor, resolution, power, and bidirectional (recording and stimulation) capabilities of various miniaturized optical microscopes.

[†]Estimated from figures and scale bars. [‡]Estimated from image sensor data sheet. Other power-hungry blocks such as the LED power consumption are rarely reported. The total system power consumption is underestimated and is likely to be much greater. [§]Measured total power consumption including image sensor and LED power.

	Ref ⁶⁵	Ref ⁶⁶	Ref ⁶⁷	Ref ⁶⁸	Ref ¹	Ref ⁶⁹	This Work
Name	Time-Resolved	Time-Resolved	Time-Resolved	Time-Resolved	Time-Resolved	Time-Resolved	Intensity
Array Size	160×120	256×256	512×512	256×256	160×160	128×120	192×256
Technology	350nm	130nm	180nm	90/40nm	130nm	90/40nm	130nm
Pitch (Fill Factor)	15um, 21%	16um, 61%	16um, 10.5%	9.2um, 51%	30um, 5%	8um, 45%	25um, 11.5%
PDP (Excess Bias)	--	39.5%, 3V	50%, 6.5V	23%, 3V	12%, 3V	12.6%, 3V	9%, 1.5V
DCR (Excess Bias)	580cps, 3V	40cps, 1.5V	75cps, 6.5V	20cps, 1.5V	26cps, 3V	15cps, 1V	17cps, 1.5V
Frame Rate (fps)	486 (8b)	100,000 (1b)	274 (8b)	30 (8b)	125 (10b)	15	200/400
Sensitivity	1.06 fW/pixel	0.06 fW/pixel	2.00 fW/pixel	0.07 fW/pixel	1.65 fW/pixel	0.10 fW/pixel	0.63 fW/pixel
Dynamic Range	74 dB	64.4 dB	48 dB	120 dB	60.2 dB	72dB / 126 dB	60.2 dB
Power	157mW	--	26.7 mW	77.6 mW	40 mW	10 mW	20 mW

Table S2. SPAD characterization. Table compares imaging modality (time-resolved or intensity), array size, technology, pixel pitch, PDP, DCR, frame rate, dynamic range, and power consumption with current state-of-the-art SPAD imagers.

Materials	Young's Modulus	Ref
Parylene C	2.8 GPa	Ref ⁷⁰
Polyimide	2.07-2.76 GPa	Ref ⁷¹
CMOS (0.5um three metal n-well CMOS process)	~60GPa	Ref ⁷²
Absorption filter (~20um KMPR photoresist)	6.5–7.5 GPa	Ref ⁷³
Interference filter	e.g., 0.9085GPa	Ref ⁷⁴

Table S3. Mechanical properties of materials. Table compares the Young's modulus of materials in the final packaging stack up of the SCOPe system.

Optical Parameter	Ref ⁷⁵ – 470nm	Ref ⁷⁶ – 640nm
Scattering coefficient (μ_s)	10mm ⁻¹	9mm ⁻¹
Absorption coefficient (μ_a)	0.21mm ⁻¹	0.02mm ⁻¹
Anisotropy (g)	0.9	0.89
Index of refraction (n)	1.38	1.36

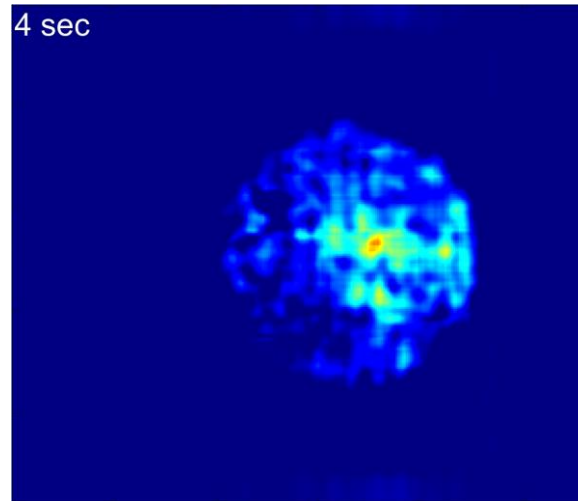
Table S4. Monte Carlo Light Propagation Modeling – Optical Parameters. Table includes the scattering coefficient, absorption coefficient, anisotropy factor, and index of refraction at 470nm and 640nm light.

	Mouse Exp 1	Mouse Exp 2	NHP Exp 1
# Of Blue LEDs	6	4	22
Optical Power per Blue LED	4.5 μ W	3 μ W	9 μ W
Electrical Power per Blue LED	62 μ W	41 μ W	123 μ W
Blue LED EQE	7.3%		
# Of Red LEDs	-	1	-
Optical Power per Red LED	-	193 μ W	-
Electrical Power per Red LED	-	12.25mW	-
Red LED EQE	1.6%		

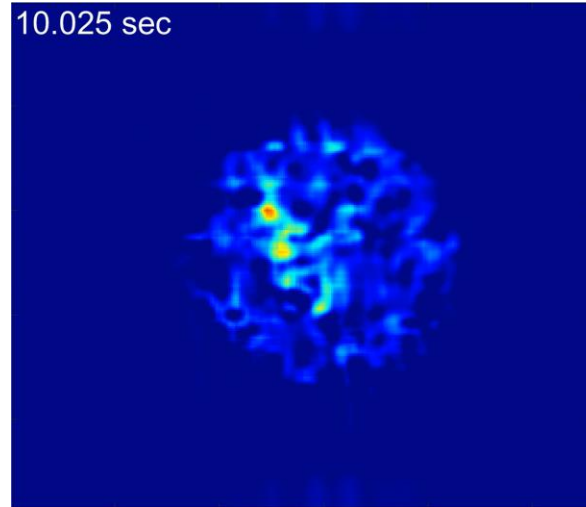
Table S5. LED optical powers used *in vivo*. Table compares the total optical power emitted by each blue and red LED during the *in vivo* optical recording and stimulation experiments.

Recording	LED Voltage	Comments
0-5	0	Dark
6-9	2.48	Simple Touch
10-11	2.49	Simple Touch
12	2.5	Simple Touch
13	2.48	Baseline
14-22	2.48	Contralateral
23-26	2.48	Ipsilateral
27-28	2.48	Baseline
29-33	2.48	Ipsilateral
34-35	2.48	Contralateral
36-40	2.48	Simple Touch
41	2.48	Baseline
42-43	2.48	Arm-restrained baseline

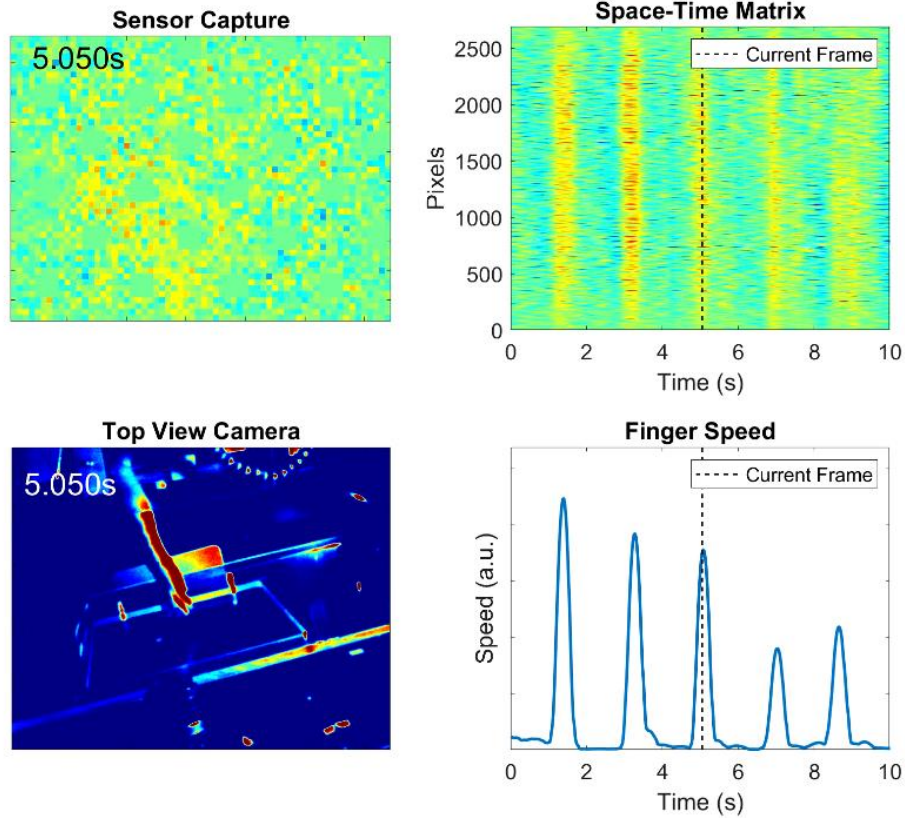
Table S6. Recordings in nonhuman primate experiment. Table includes the blue LED voltage supply along with the experimental task type (dark, simple touch, baseline, contralateral, ipsilateral, arm-restrained baseline) for the nonhuman primate experiments.



Movie S1. In vivo mouse experiment – electrical stimulation and optical recording. Electrical bi-phasic stimulation pulses with 100 μA applied with flexible electrodes. $\Delta F/F$ images reconstructed at a depth of 100 μm . Color scale is 0-0.2 $\Delta F/F$.



Movie S2. In vivo mouse experiment – optical stimulus and optical recording. Optical stimulation pulses with 9.65 mW/mm^2 applied with on-chip red μLED for four frames ($\sim 100 \text{ ms}$). Neural responses ($\Delta F/F$) are imaged for 400 frames ($\sim 10 \text{ s}$) using illumination from four on-chip blue μLEDs and reconstructed at a depth of $100 \mu\text{m}$. Color scale is 0-0.2 $\Delta F/F$.



Movie S3. In vivo NHP experiment – behavioral stimulus and optical recording. (Top left) Processed 2D image sensor capture at an example time sample of 5.050 s. (Top right) Space-time matrix representation of the image sensor capture with the 8x8 binned pixel number of the y-axis and the time sample on the x-axis. The black dotted line corresponds to the image capture frame in the top-left panel. (Bottom left) Top view camera frame synchronized to the image capture frame at an example time sample of 5.050 s. (Bottom right) The finger tracking result from the DLC pipeline converted into speed with the black dotted line corresponding to the time sample of 5.050 s.

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