Supplementary Note

The closed-loop software architecture

An overview of the hardware, electronics and software for implementing our closed-loop all-optical strategy is shown in **Supplementary Fig. 1a**. The custom closed-loop software toolkit consists of three parts: the closed-loop interface (**Supplementary Fig. 1b**; written in VB.net), the sensory stimulation control software (**Supplementary Fig. 1c**; written in Matlab); and the SLM control software (**Supplementary Fig. 1d**; written in C++, and based on the Blink SDK provided by Meadowlark Optics). The closed-loop interface (**Supplementary Fig. 1b**) analyses the raw data acquired by the two-photon microscope (Bruker Corporation) on-the-fly and communicates with the microscope control software (Prairie View, Bruker Corporation), the SLM control software and the sensory stimulation control software via TCP/IP sockets. The user interface (UI) enables users to select regions of interest (ROIs), register frames and monitor calcium signal traces online, specify experimental protocols, and save recordings. The UI can work in two modes: display-on and display-off. In the display-on mode, the current calcium image and the calcium traces extracted from the ROIs are displayed on the UI. The display panels are typically turned off during the experiment to maximize high-speed performance.

Three types of experiments can be performed with this toolkit:

1) *Activity clamp*: The user specifies the target $\Delta F/F$ thresholds, baseline frames and clamping durations. Phase masks for generating spots on the cell(s) of interest are uploaded to the SLM before the experiment starts. Photostimulation pulses are triggered if the online recorded $\Delta F/F$ falls below the threshold.

2) *Boost sensory response*: The user specifies the type of ‘the user specifies the strength of whisker deflection stimuli, number of baseline frames and the level of activity threshold in the closed-loop interface. In the sensory stimulation control software, the user can specify different types of voltage waveforms for driving a piezo stimulator. Whisker stimuli can be triggered manually or in a sequence defined by the user. The stimulation command in the socket specifies the type of sensory stimulation (e.g. strong deflection, weak deflection, or long sinusoidal wave for finding sensory responsive cells). The corresponding voltage signal is sent to the piezo controller upon receiving a command (**Supplementary Fig. 1c**). Phase masks that will generate

single spots on the cell(s) of interest are uploaded to the SLM before the experiment starts. Photostimulation will be sent to the cell(s) of interest if their sensory-evoked activity did not pass the activity threshold within a user-defined timeout window after the delivery of sensory stimuli.

3) *Trigger-targets*: The phase masks that will result in the photostimulation pattern with individual beamlets on all possible combinations of the groups of trigger cells are loaded into the buffer of the SLM control software (**Supplementary Fig. 1d**). Each phase mask was indexed as

$$PhaseMask\_Idx = \sum_{i \in P} 2^i \quad (1)$$

where *P* is the set containing the indices of the target groups that this phase mask will direct the photostimulation beamlets onto.

The user selects one or multiple 'trigger cells' and provides phase masks for photostimulating the 'target cells' that are assigned to each trigger cell. The activity threshold of each trigger is updated at every frame. A buffer of size *N* (*N* = 60 in our experiments) was assigned to each trigger to record the fluorescence intensity value, $F(i, n)$, $0 < n < N$, in the *N* frames before the current frame. The activity threshold for the i^{th} trigger ROI in the j^{th} frame is defined as

$$T(i,j) = M(i,j) + 2 \times SD(i,j), \quad (2)$$

where $M(i, j)$ and $SD(i, j)$ are the average value and the standard deviation of the current intensity values in the buffer for trigger *i*, respectively. *M* and *SD* are calculated by a one-pass algorithm[1] to increase computational speed:

$$M(i,j) = M(i,j-1) + (F - F(i,n))/N \quad (3)$$

$$F_{sq_{sum}}(i,j) = F_{sq_{sum}}(i,j-1) - F(n)^2 + F^2 \quad (4)$$

$$SD(i,j) = ((F_{sq_{sum}}(i,j) - N \times M(i,j)^2)/(N-1))^{1/2} \quad (5)$$

where $F(i, n)$ is the intensity value in the buffer to be replaced by the current intensity value F recorded from ROI i. Index n increments after every loop and is reset to 1 when it reaches N. If $F > T(i, j)$, the targets assigned to trigger i will be included in the photostimulation targets by adding 2^{i-1} to the phase mask index. The phase mask index is sent to the SLM control software after all trigger ROIs have been checked, and is then reset to 0.

The phase mask on the SLM will be updated if the received index is different from the index of the current phase mask. An echo is sent to the closed-loop interface when this process is complete, to avoid triggering photostimulation before the phase mask has been updated.

In all experiments, the spiral size, revolutions (number of cycles in a spiral) and duration of the photostimulation protocols are defined in Prairie View (Bruker Corporation). The access to the raw image data stream depends on PrairieLink (Bruker Corporation). The phase masks are uploaded to the SLM using the Blink_SDK dll (Meadowlark Optics). Analogue voltage outputs were generated using NI-DAQmx dll (National Instruments). Software platforms used in the closed-loop package: VB.net, Visual Studio 2013 (64 bit) and Matlab (2016a). In principle, the code could be adapted for other software environments, such as ScanImage[2].

The original code, together with detailed instructions and sample data can be found at the following Github link: https://github.com/alloptical/ClosedLoop.

REFERENCES

1. Chan, T.F., Golub, G.H. & LeVeque, R.J. *The American Statistician* **37**, 242-247 (1983).
2. Pologruto, T.A., Sabatini, B.L. & Svoboda, K. *BioMedical Engineering OnLine* **2**, 13 (2003).