**Synchronization of Stimulation Window to Imaging Frames (e.g. Laser Gate)**

Since two-photon excitation by the photostimulation laser yields fluorescence that prevents the PMT from recording neural activity, we have developed an electronic circuit to address this issue by forcing the photostimulation laser to illuminate neurons only as the resonant galvomirror reverses course, on either side of the imaging window. The electronic circuit is shown in Supplementary Fig 14a.

We take as input the square feedback signal from the resonant galvomirror. This TTL pulse (0-5V) precisely changes state when the galvomirror changes its course, and oscillates at the 8kHz resonant frequency (for effective 16kHz duty-cycle of photostimulation, since photo-stimulation occurs twice per 2P imaging line). Our circuit uses high speed operational amplifiers (OP07CP, Texas Instruments) and first amplifies the input signal with a follower circuit to avoid perturbing the feedback input signal. The amplified signal is fed into an RC circuit, (C = 2 μF, R = 170Ω). The resulting analog signal in the capacitor, VF, (Supplementary Figure 14b) reaches peak high and low value anytime the galvo mirror reverses its course. This signal is sent to two threshold detection circuits. The first output, TL, returns a logical 1 (5V) anytime VF<VL. Similarly, the second output TH, returns a logical 1 anytime VF>VH. The threshold values VH, and VL can be independently tuned with a 10kΩ potentiometer to adjust the left and right limits of the imaging window. A TTL OR gate (SN7432) combines both threshold detections, and the output signal, oscillating at 16kHz is used to control the gate of the photostimulation laser, and prevents photostimulation to happen while the resonant galvo mirror scans and measures fluorescence within the boundaries of the imaging window.

This circuit may be built using the provided circuit board (.fzz file) and off-the-shelf electronic components.

