Photoconductive stimulation of neurons cultured on silicon wafers

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Photoconductive stimulation allows the noninvasive depolarization of neurons cultured on a silicon wafer. This technique relies on a beam of light to target a cell of interest while applying a voltage bias across the silicon wafer. The targeted cell is excited with minimal physiological manipulation, and, therefore, long-term modulation of activity patterns and investigations of biochemical mechanisms sensitive to physiological perturbations are possible. Ideologically similar to transistor-based neuronal interfaces, the photoconductive-stimulation method has the advantage of being able to extracellularly excite any neuron in a network regardless of its spatial position on the silicon substrate. This protocol can be easily implemented on a conventional reflected-light fluorescence microscope using materials and resources that are readily available. Time requirements are comparable to standard cell-culture and electrophysiology techniques. When combined with fluorescence imaging of various molecular probes, activity-dependent cellular processes can be dynamically monitored.

INTRODUCTION

Manipulating cell excitation is central to investigating the physiological processes and functions of excitable tissues. A variety of electrical and chemical methods for cell stimulation have been devised over the years. The classical approaches involve stimulation with electrodes or chemical modulation of voltage-gated and ligand-gated ion channels. In recent decades, interfaces between silicon technology and manipulation of living cells have opened new methodologies for achieving noninvasive extracellular stimulation. When combined with dissociated neuronal cultures or organotypic preparations as model systems, the silicon interface provides a powerful tool for examining neuronal network behavior[1–4]. Transistor array interfaces are used to stimulate and acquire detailed measurements of membrane-potential changes of neurons positioned over the transistor element. However, the utility of the transistor array is constrained by the fixed spatial position of the transistor elements. In order to interface a complex neural network with a method that harnesses true random-access capability for stimulation of neurons, we developed a light-addressable technique[5]. This method takes advantage of the property of silicon wafer, where light generates a charge carrier upon concurrent application of a bias voltage across the wafer[6]. By targeting the light to a region of interest, local photocurrent can extracellularly stimulate neurons in the light path. The spatial resolution of photoexcitation and charge-carrier generation in silicon wafers is estimated to be less than 100 μm (ref. 7); thus, single-cell excitation can be elicited by illuminating an isolated neuronal cell body.

A number of optical methods for neuronal stimulation have been described. For instance, cell-specific expression of genetically encoded light-sensitive controllers of membrane voltage can be used to manipulate cell excitability[8]. In addition, lasers can be used to evoke synaptic responses by uncaging either the extracellular neurotransmitters or the intracellular calcium at nerve terminals to promote neurotransmitter release[9,10]. Whereas these techniques are suitable for some applications, our protocol is unique amongst light-directed cell-excitation methods. It is simple to implement and highly cost-effective. It obviates the complications associated with expressing exogenous roteins, while permitting spatially selective excitation of an area within 100 μm^2 that is not easily achievable with conventional extracellular-field stimulation. The basic photoconductive-stimulation protocol described here has allowed the patterned stimulation of neural networks[11,12] and the study of activity-dependent synapse formation by observing the remodeling of synaptic cytoskeleton[5]. The system can be modified to integrate a laser beam and an acousto-optic deflector, with which a complex spatiotemporal-stimulation pattern can be generated to study detailed network properties[13]. Furthermore, this method is applicable to studying excitable behavior of non-neuronal cell types, such as muscle and secretory cells.

The primary limitation of this stimulation protocol relates to the optical methods used to detect induced activity, for example calcium-imaging or voltage-sensitive dyes. Extended presence of these compounds and their photoexcitation could interfere with the cellular physiology of the neurons. However, once the stimulation protocol is calibrated using such dyes, the neurons can be reliably fired for extended periods of time and the stimulation procedure becomes routine. Of critical importance is the health of the neurons, similar to electrophysiology recordings. Neuronal cultures that withstand electrode recordings are also ideal substrates for photoconductive stimulation.

MATERIALS

REAGENTS

- 10× Hanks balanced salt solution (HBSS; Invitrogen, cat. no. 14185-052)
- 1 M HEPES (Sigma, cat. no. H-7523)
- 1 N NaOH
- Sorbitol (Sigma)
- Basal Medium Eagle (BME; Invitrogen, cat. no. 21010-046)
- D-glucose (Sigma)
- 100× glutamine (Sigma, cat. no. G-8540)
- Na-pyruvate (Invitrogen, cat. no. 11360-070)
- FBS batch tested (Hyclone, cat. no. SH30397.03) ▲ **CRITICAL** We screen several lots (six to eight) of FBS for optimal viability of glia and neurons from various sources (e.g., Hyclone, Sigma, Invitrogen). The best lot is purchased in bulk and stored at –80 °C
- Penicillin/streptomycin (pen/strep; Invitrogen, cat. no. 15070-063)
- B27 supplement (Invitrogen, cat. no. 17504-044)
- $CaCl_2$ + L-cysteine (Sigma)
- EDTA (Sigma)
- Papain (Worthington Biochemical Corporation, cat. no. LS003126)
- NaCl (Sigma)
- 2.5 M KCl
- 4.9 M $MgCl_2$
- Poly-D-lysine: 1 mg ml^{-1} stock in H_2O (Sigma, cat. no. P7280, 5 mg) ▲ **CRITICAL** The solubilized aliquots should be kept no more than 3 months at –80 °C
- Laminin (Sigma, cat. no. L2020, 1 mg ml^{-1})
- Fluo-4 acetoxymethyl ester (Fluo-4 AM) calcium indicator (Molecular Probes)
- FM styryl dyes (Molecular Probes)
- Epoxy resin: commonly available from hardware stores, a clear epoxy resin that sets in 5 min is sufficient

EQUIPMENT

- Silicon wafers: 385–410 μm thickness, P-type, 1-0-0 orientation, 10 Ω-cm single-side polished (Silicon Quest)
- Platinum wire: 0.5 mm diameter, 99.9% pure (with iridium; Sigma-Aldrich, cat. no. 267228)
- Falcon 351008 cell-culture dish: 35 × 10 mm (Falcon)
- Soldering iron: for connection to electrodes
- Upright microscope: reflected-light fluorescence illumination attachment is necessary. We use BX50WI, BX51WI or BX61WI (all from Olympus) fitted with a mercury lamp
- Image-acquisition system: a cooled charge-coupled device (CCD) camera attached to the upright microscope
- Brightfield half-mirror cube unit (U-MBFL, Olympus)
- Grass S88 Stimulator (Grass Telefactor)
- Sprague-Dawley newborn pups (Charles River)

REAGENT SETUP

Dissection solution 100 ml HBSS (10× stock), 2.4 g HEPES. Adjust pH to 7.2 with NaOH (1 N) and add water to 1,000 ml. Adjust osmolarity to 310 mOsm with sorbitol (~0.182 g $mOsm^{-1}$ l^{-1}). Filter sterilize into 2× 500-ml bottles using a 0.2-μm filter. Store at 4 °C.

Growth medium 42.5 ml BME, 0.3 g D-glucose, 500 μl of 200 mM glutamine (aliquoted and frozen at –20 °C), 500 μl of 1 M HEPES (aliquoted at –20 °C), 500 μl of 100 mM Na-pyruvate, 2.5 ml FBS (aliquoted and stored at –20 °C), 1 ml pen/strep (aliquots stored at –20 °C), 1 ml B27 supplement (aliquots stored at –20 °C).

Enzymatic buffer 30 μl of 150 mM $CaCl_2$, 100 mM L-cysteine, 2 ml dissection solution, 20 μl EDTA (50 mM), 80 μl papain (2× crystalline suspension). Heat to 37 °C for 30 min and filter sterilize with a 0.2-μm filter.

Extracellular bath solution (EBS) 7.89 g NaCl (135 mM), 3 ml of 1 M $CaCl_2$ (3 mM), 2 ml of 2.5 M KCl (5 mM), 408 μl of 4.9 M $MgCl_2$ (2 mM), 1.8 g D-glucose (10 mM), 2.4 g HEPES (10 mM). Add $CaCl_2$ to the solution last, adjust pH to 7.3 with NaOH and add water to a final volume of 1,000 ml.

Borate buffer for the silicon-wafer preparation 1.24 g boric acid (molecular weight, 61.83), 1.90 g sodium tetraborate (molecular weight, 381.37), add to 400 ml H_2O and adjust pH to 8.5 with NaOH.

EQUIPMENT SETUP

Brightfield half-mirror cube unit This allows brightfield observation of neurons grown on silicon chips using reflected-light illumination from a high-intensity light source, such as the mercury lamp.

Stimulation device While a variety of designs can be used for construction of the stimulation device, the following is a simple and effective version. The stimulation chamber consists of three components: (i) a dish to hold the bath solution; (ii) a square frame to place the wafer on, thereby isolating two chambers (an inner chamber beneath the wafer and an outer bath chamber); and (iii) two electrodes, one for the inner chamber and another in the bath solution (**Fig. 1a**). This will function effectively for the observation of a single wafer under an upright microscope.

Current source Any pulse generator capable of producing a 2-ms pulse of 0 to +5 V can be used to supply the chip with the desired frequency. Although current requirements are low, ~500 mA should be available to compensate for variations in buffer conductivity and stimulation-chamber design. Measurement of the current draw during stimulation indicates that ~20 mA is required at lower frequencies (1–2 Hz), whereas up to 120 mA is required at higher frequencies (30 Hz). However, note that the actual current flow through the wafer at the lighted spot is in the order of sub-mA. A Grass S88 Stimulator is a common and convenient pulse-train source of sufficient power. Alternatively, more complex stimulation patterns can be achieved using computer-controlled electronics. Note, however, that transistor–transistor logic (TTL) circuits have insufficient power to drive the device, and therefore should be used only to trigger a power source.

PROCEDURE

Construction of the stimulation device

1| To construct the device, the bottom half of a common cell-culture dish serves as a practical container. The frame to support the wafer can be made from ~2.5-mm thick Plexiglas by first cutting it into a square and then removing the smaller center square piece (**Fig. 1a**). The electrodes are formed by bending the platinum wire as shown in **Figure 1a**, and then drilling a small hole in the dish to mount them.

▲ **CRITICAL STEP** Note especially the configuration of the inner-chamber platinum wire, which is bent to increase the surface area in contact with the inner-chamber solution. The platform and wires should be glued into place using epoxy resin. Wire leads are then soldered to the platinum, taking care not to melt the plastic.

■ **PAUSE POINT** The stimulation device has no special storage requirements and can be stored indefinitely until needed.

Silicon-wafer preparation

2| Silicon comes as circular plates described as either 4 or 6 inches in diameter (~100 or 150 mm, respectively). Cut the wafers by a laser or diamond saw into 1-cm squares. Alternatively, cut the squares manually (and relatively easily) by scribing a line on the polished surface of the wafer with a diamond pen and cracking the wafer along the score. Gloves should be worn at all times when handling the silicon. The sample chamber accommodates imperfectly shaped wafers because the rim of the square frame

that serves as the platform for the silicon chips in the sample chamber is several millimeters in thickness, and its outer edge is slightly larger than the wafer. Of main importance is the ability of the wafers to fit in the wells of a 24-well plate.

3| As purchased, the silicon wafers are sufficiently clean to use without further processing. If foreign material is introduced to the surface during processing, it can be gently cleaned with 70% ethanol (vol/vol) on a cotton swab.
▲ **CRITICAL STEP** Care must be taken not to scratch the surface.

4| Oxidize the silicon-wafer surface by heating the wafers in atmospheric air. To do so, place the wafers on an aluminum plate (~5 mm thick), polished side up, and place on a high temperature (~700 °F or ~370 °C) hot plate in a clean environment. Remove the plate from the heat after 5 min and allow to cool. The natural oxide layer formed on the silicon wafer as purchased might be sufficient, in which case this step can be omitted. We tested silicon wafers after deliberately growing the surface oxide layer to 2, 6 and 8 nm. Cell viability and neuronal stimulation were poor in all three conditions, such that the optimal thickness of the oxide layer was below 2 nm (C. Dillon and Y. Goda, unpublished observations).
■ **PAUSE POINT** Place the treated wafers in 70% ethanol (vol/vol) for storage until use.

5| Place cut wafers in 24-well plates in a biosafety hood. Fill all wells with 70% ethanol (vol/vol; ~1 ml per well), lifting each wafer briefly off the bottom of the well to allow the ethanol to reach the unpolished side of the wafer. Aspirate the 70% ethanol (vol/vol) and replace with 100% ethanol (vol/vol, dehydrated). Allow the silicon wafers to soak in 100% ethanol (vol/vol) in the hood for 1 h, then thoroughly aspirate the ethanol off from both sides of the wafer.
▲ **CRITICAL STEP** Allow 10 min to ensure that the wafers are completely dry.
■ **PAUSE POINT** Plates can be prepared hours in advance.

6| Prepare working solution of poly-D-lysine, 1 μg ml^{-1}, by diluting the stock in borate buffer. Apply ~400 μl poly-D-lysine solution per well and allow to sit at room temperature (22–25 °C) overnight.

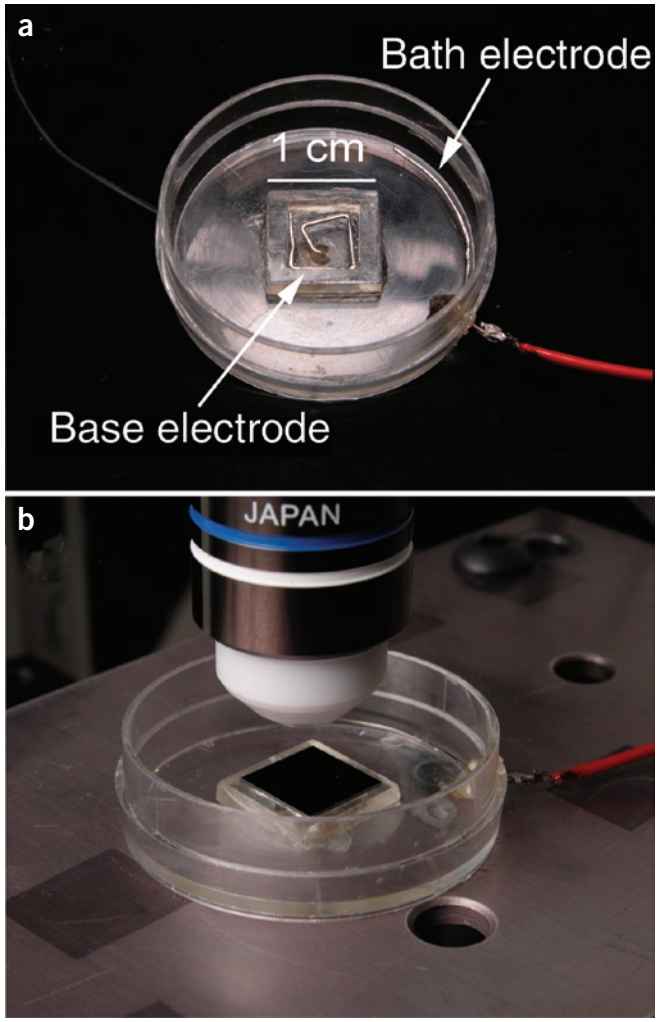

Figure 1 | Photoconductive-stimulation device. (**a**) The device shown is assembled from common laboratory materials and a platinum wire. In the bottom of a 35-mm culture dish, a platform upon which the silicon wafer sits is cut from Plexiglas into a square frame shape and glued in place. The surface of the Plexiglas frame should be ~2–3 mm in width to accommodate variations in the size of the wafers. Holes are drilled at the bottom and the side of the dish to thread the platinum electrodes, which are then also glued down. (**b**) Image showing the device mounted under an Olympus BX61 microscope, with a wafer on the Plexiglas platform.

7| Rinse excess poly-D-lysine in borate buffer five times for 5 min each. Do not dry the poly-D-lysine-coated wafers; proceed immediately to the laminin coating.

8| Dilute laminin to 1 μg ml^{-1} in PBS, and apply to silicon wafers.
▲ **CRITICAL STEP** Laminin application must be timed with the cell preparation to allow treatment of the chips for 3–4 h prior to cell plating. Altering this time will result in altered morphology or growth characteristics of the neurons.

Preparing dissociated neurons for culturing on silicon

9| Experimental design ethical review statements must be obtained for this procedure. Remove hippocampi from eight Sprague-Dawley newborn pups (sufficient for 48 wafers) and transfer to dissection solution in a small petri dish placed on ice. Chop hippocampi using the tip of a glass Pasteur pipette, breaking each hippocampus into approximately four pieces, to allow better enzyme access. Transfer pieces of tissue with minimal volume of dissection solution to pre-warmed, filtered protease solution.

Incubate for 30 min at 37 °C in a CO_2 incubator, with the top of the tube loosened.

10| Heat the tips of two standard Pasteur pipettes to reduce the size of the tip opening to one-third and one-quarter of the original diameter, respectively. Heat the tip of a third pipette to remove the sharp edge from the tip without substantially altering the bore size. Retrieve hippocampal tissue from the incubator and aspirate excess protease solution. Wash the hippocampi three times in warm culture medium using the polished pipette, and then re-suspend in 1 ml medium. Triturate cells successively through the reduced bore pipettes, passing the full cell volume each time, ~10 times, first with a larger diameter tip pipette followed by a smaller diameter tip pipette.

11| Count dissociated cells on a hemocytometer, and then dilute the cell suspension in medium to achieve the desired plating density for the appropriate number of wafers. We routinely use 5×10^4 cells in 700 µl culture medium per 1 cm^2 chip in a single well of a 24-well plate.

12| Rinse excess laminin thoroughly from both sides of the wafers with three washes of PBS, with no delay between solution changes. Then immediately plate neurons.

▲ **CRITICAL STEP** Neurons need to be seeded immediately after the rinse without drying out the surface of the silicon wafers. Cultures are grown in the incubator undisturbed for 3 d, at which time the medium is refreshed by removing one-half the volume and replacing it with fresh warmed medium.

■ **PAUSE POINT** Let neurons grow for 2–3 weeks until stimulation and imaging is performed.

Photoconductive stimulation of neurons

13| Transfer wafers with cultured neurons from the incubator to warm EBS (37 °C) in a small culture dish. Meanwhile, coat the surface of the square Plexiglas platform of the stimulation chamber with vacuum grease and place the chamber on a microscope stage (**Fig. 1b**). Fill the chamber with warm EBS, ensuring that the inner chamber is also completely filled without air bubbles. Place the wafer squarely on the platform using forceps.

▲ **CRITICAL STEP** Gently press the wafer in the center with the forceps to ensure a tight seal on the platform.

14| When first using this stimulation technique, it is useful to pre-incubate the wafers with cultured neurons with a calcium indicator such as Fluo-4 AM, to visualize the firing of neurons. To use Fluo-4 AM, load the cells by incubating silicon wafer cultures for 15–60 min in warm EBS containing 1–5 µM dye in a small petri dish, then mount the wafers on the stimulation chamber as indicated above.

15| Use a light meter to quantitatively determine the intensity of illuminating light needed for firing the neurons. Place the sensing device under the objective, and adjust the light output until ~60 lm is transmitted. This should be sufficient for stimulation with a 5-V pulse. Alternatively, for a simple but effective empirical method for calibrating the light intensity, start with an intermediate light level. Engage appropriate neutral-density filters, and open both field and aperture iris diaphragms in the reflected-light illumination tube. Then, illuminate the chip while applying a brief pulse train to the stimulation chamber (5 s at 10 Hz, 5 V, with pulse width of 2 ms). Remove neutral-density filters until bubbles appear on the surface of the silicon when the pulse train is applied. Over-stimulation will necessitate moving to a new area of the wafer.

▲ **CRITICAL STEP** Extreme over-stimulation will destroy the cell layer. The efficacy of stimulation is highly dependent on light intensity but not critically on the wavelength over the range commonly used for white light or fluorescence observation. According to the optical readout of neuronal excitation used, standard fluorescence-filter sets or the brightfield half-mirror cube unit are sufficient for stimulation.

16| After determining the intensity level at which the illuminating light causes the appearance of bubbles, reduce the light-intensity level by 50% using appropriate neutral-density filters. Stimulation at this intensity should trigger action potentials in neurons within view, as observable by the flashing of the Fluo-4 indicator, without incurring cell damage (**Fig. 2**). Concurrent electrophysiological recording

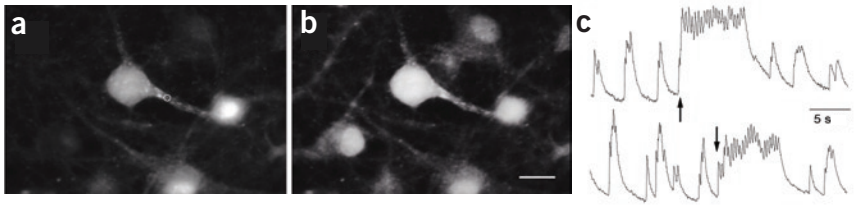

Figure 2 | Photoconductive excitation and calcium imaging in a Fluo-4-loaded neuron. (**a**,**b**) Sample frames from a Fluo-4 calcium-imaging video of spontaneously active neurons before (**a**) and during (**b**) photoconductive stimulation. Depolarization is detected by an increase in fluorescence signal. Scale bar, 20 µm. (**c**) Time traces of Fluo-4 calcium-signal intensity (arbitrary units) in the proximal dendrite of a neuron (circled region in **a**). Arrows mark the beginning of stimulation at 2.5 Hz for ~10 s. Note that spontaneous activity is apparent both before and after stimulation.

from the targeted neuron can also be used to test the threshold light intensity to elicit excitation (**Fig. 3**). In the example shown, voltage-pulse application across the wafer in the dark does not trigger synaptic responses, whereas concurrent illumination elicits robust evoked synaptic currents.

17| Restrict illumination to the cell body of a single neuron by closing down the field-iris diaphragm of the epifluorescence light source. This will drive targeted excitation of the illuminated neuron, provided that there are no other neurons within 100 µm. To confirm the specificity of stimulation to the targeted area, we routinely use styryl dyes, such as FM1-43, to label synaptic output of the stimulated neuron in the presence of postsynaptic-receptor blockers in the bath solution[5]. Synaptic labeling is confined to axons of the excited cells within the light path.

18| Maintain cultures during stimulation using a warmed perfusion system or by manually refreshing the EBS.

19| Image neurons before, during and/or after stimulation for markers of interest. This is conveniently accomplished with the epifluorescence light source used for stimulation and a CCD camera with appropriate sensitivity for the optical probe being used.

20| Although not recommended, used silicon wafers can be recycled once by briefly soaking in HCl/MeOH (1:1) mix, followed by three washes in deionized water, and then two washes in 70% ethanol (vol/vol).

■ **PAUSE POINT** Place the treated wafers in 70% ethanol (vol/vol) for storage. Proceed to Step 5.

● TIMING

Step 1, 1 h; Steps 2–4, 3 h; Step 5, 1.5 h; Steps 6–8, 12–24 h; Steps 9–12, 2 h; Steps 13–16, 2 h; Steps 17,18, variable according to individual experimental design; Step 19, 30 min.

? TROUBLESHOOTING

Troubleshooting advice can be found in **Table 1**.

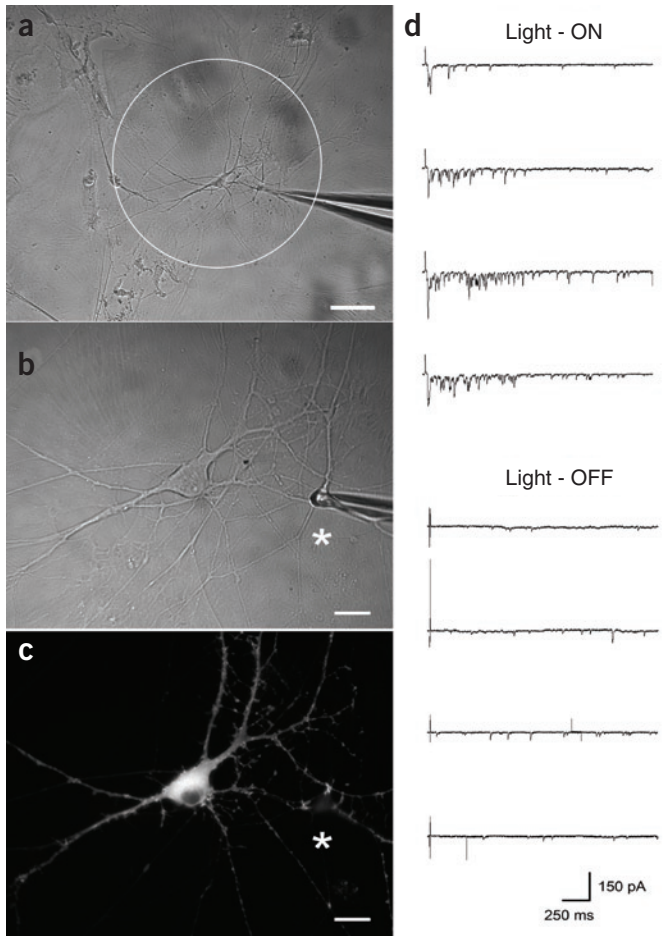

Figure 3 | Excitatory synaptic currents elicited by photoconductive stimulation in 2-week-old cultured neurons. (**a**–**c**) A voltage pulse (5 volts, 2 ms) was applied across the silicon wafer in the dark or with light (circled region in **a**) while whole-cell patch clamping the neuron (asterisk). The pyramidal neuron to the immediate left of the recorded cell was expressing GFP-actin (**c**). Scale bars: **a**, 80 µm; **b**,**c**, 20 µm. (**d**) The voltage pulse applied with the light on, triggered synaptic currents. As expected from the presence of at least three neurons within the targeted illuminated region, robust recurrent synaptic activity was apparent (upper traces). The same voltage-pulse application in the dark did not trigger synaptic responses, and only sparse spontaneous events occurred (lower traces).

TABLE 1 | Troubleshooting table.

PROBLEM	CAUSE	SOLUTION
Neurons do not fire.	Poor viability of the neurons.	The most important factor for successful photoconductive stimulation of neuronal cultures is the health of the neurons. Clean, appropriately treated silicon wafers support the healthy growth of the cultures. As often experienced with studies involving conventional primary neuronal cultures, many problems can be attributed to the fitness of the neurons themselves.
	Lack of effective seal between the silicon wafer and its platform.	The stimulation device should be thoroughly cleaned between experiments, and fresh vacuum grease applied carefully and evenly. Misalignment or an uneven surface will cause current leakage and prevent successful firing of neurons.
	Excessive growth of glial cells.	We routinely employ co-cultures of neurons and glia. Suboptimal culture conditions can result in excessive glial-cell growth. Neurons growing on top of thinly extended glial cells can be easily fired using photoconductive stimulation. However, if the distance between the neuron and the surface of the silicon begins to exceed ~100 µm, firing the neuron can become difficult.

TABLE 1 | Troubleshooting table (continued).

PROBLEM	CAUSE	SOLUTION
	Insufficient light or current intensity.	It is rarely the case that light intensity is insufficient to alter the conductivity of the silicon effectively; however, at longer wavelengths (e.g., a rhodamine-filter set) the full lamp-light intensity might be needed. It is more likely that insufficient current is actually reaching the surface of the silicon. If this occurs, increase the amplitude of the voltage pulse or check for sources of current leakage.
	Gas buildup underneath the silicon wafer.	As with any submerged electrode, excessive current will produce electrolysis of water, which can interfere with the homogeneous contact of the solution and the lower surface of the wafer. If the neurons unexpectedly stop responding to the stimulation, the situation can be immediately rectified by reversing the voltage to the device and briefly pulsing the current.
Neurons die over time.	Cytotoxicity from photodamage.	As with any live-cell observation experiment, exposure to illuminating light should be kept to a minimum. Determine the minimum light intensity required for successful stimulation using either calcium indicators or direct electrophysiological recordings of the targeted neuron. This procedure should be repeated at the start of each new batch of silicon wafers, or upon changing a significant experimental parameter.
	Perfusion of EBS solution.	The typical volume of the bath solution in the device described here is ~5 ml, and replacing it with fresh solution every 15 min is usually sufficient to allow several hours of successful activity from the cultures. However, a perfusion system should be used if experimental temperature needs to be above ambient, or if long recordings are desired.

ANTICIPATED RESULTS

The primary function of the photoconductive-stimulation device described here is for the acute, noninvasive stimulation of neurons from dissociated hippocampal co-cultures. Successful depolarization of the cells will elicit an action potential, which can be detected by optical imaging of a fluorescent calcium indicator (**Fig. 2**; **Supplementary Video 1**) or voltage-sensitive dye, or by direct electrophysiological recording. Evoked neurotransmitter release or synaptic transmission can also be monitored by FM dyes or whole-cell patch-clamp recording (**Fig. 3**). In addition to hippocampal neurons, cortical neurons and retinal ganglion cells have been successfully cultured on silicon wafers, depolarized and fired using this technique. Additionally, human embryonic kidney (HEK) cells transfected with voltage-sensitive calcium-channel subunits can be depolarized (M.A.C., unpublished data). Therefore, many excitable cell types amenable to electrical depolarization could be stimulated using this device.

A primary application of the stimulation device as implemented here is for live-cell video imaging of fluorescently tagged molecules, such as proteins fused to green fluorescent protein (GFP), and determining their response to activity. Using adequate perfusion equipment, cell cultures can be maintained and observed for hours, allowing the investigation of phenomena that occur over a longer time scale, such as synaptic remodeling.

In summary, photoconductive stimulation of excitable cells grown on silicon wafers is a protocol that provides core functionality for a variety of possible applications and devices to assist biological research. Simultaneous, multiple cell-targeting systems, as well as environmentally regulated extended-stimulation devices, are currently being developed.

Note: Supplementary information is available via the HTML version of this article.

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COMPETING INTERESTS STATEMENT The authors declare that they have no competing financial interests.

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