Application Note
LIFE SCIENCES

University College London (UCL)

Thorlabs, New Jersey

Menlo Systems, Martinsried

Neuroscience: *In-vivo* all-optical interrogation of neural networks

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Understanding neuronal communication

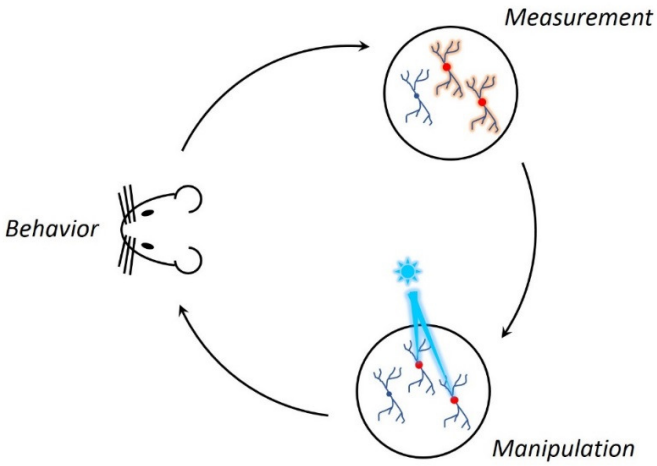

Figure 1: Schematic of all-optical physiology illustrating the interplay between behavioral experiment, imaging of activity patterns in the brain, and the manipulation of specific functionally defined neurons.

Understanding ongoing processes within the brain at the cellular level enables us to determine the physiological basis of cognition and nurtures our hopes of curing brain diseases which are nowadays difficult to medicate or are considered as immedicable. In order to understand neural processes it is important to be able to both record the activity of large numbers of neurons and to manipulate and

probe that activity to uncover functional network structure and links to cognition and behavior. In a groundbreaking experiment neuroscientists from the group of Professor Michael Häusser at University College London have succeeded in observing and controlling the activity of defined cell types at an unprecedented level. The underlying learning loop includes behavioral tasks, imaging of activity patterns in the brain, and replaying the same patterns in the identified specific functional neurons (Fig. 1). Cellular resolution functionally defined optogenetics has thus moved from being a 'dream experiment' to a real application, enabling a deeper insight into neuronal communication.

Deep tissue imaging and photostimulation

Although the basic mechanism of neural activity is well understood, the complex interconnection of specific neurons or groups of neurons, and the behavioral pattern they are linked to, require novel and interdisciplinary techniques of investigation. Häusser et al. have managed to observe the activity of a large population of single neurons and to manipulate them to determine the extent to which their action is reproducibly linked to a behavioral output.

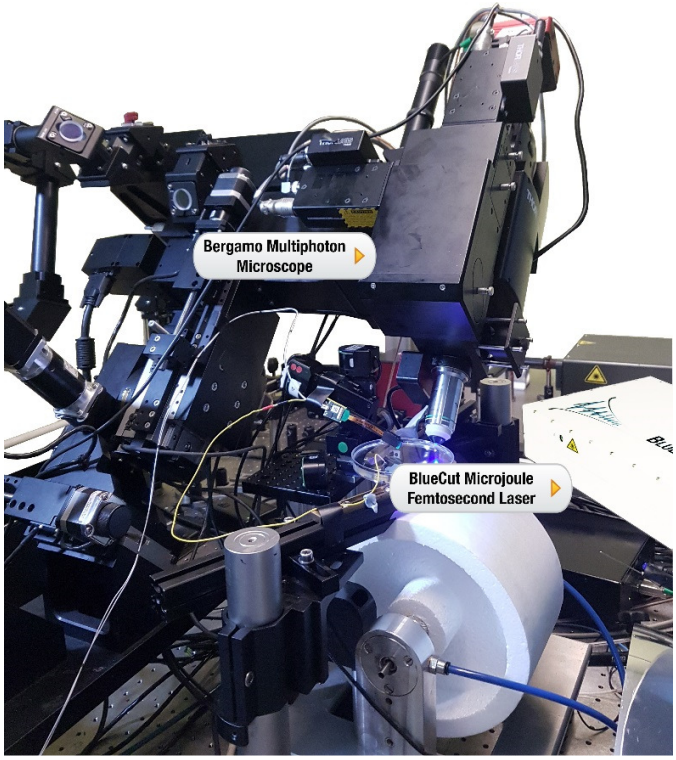

Figure 2: Experimental setup with the Thorlabs Bergamo multiphoton fluorescence microscope in rotated geometry. In the background is the stimulating BlueCut laser from Menlo Systems.

Neurons communicate with one another through electric potentials migrating as a signal along the cell membrane. The level of positively and negatively charged ions present in and around the cells control their activity. When an action potential is triggered Ca^{2+} ions flow through channels in the membrane into the neuron. Häusser et al. genetically modified the brain of a mouse, inserting two different proteins, a calcium sensitive fluorophore and a light sensitive ion channel. The calcium sensor allows microscopic visualization of neural activity, since its fluorescence is significantly increased in the presence of Ca^{2+} ions. The ion channel which is inserted into the cell membrane can be optically

triggered to cause Na^+ ions to flow into the cell, resulting in a neural action potential. The action potential then opens voltage gated Ca^{2+} channels, Ca^{2+} ions flow into the cell and increase the fluorescence, allowing the success of the stimulation to be confirmed.

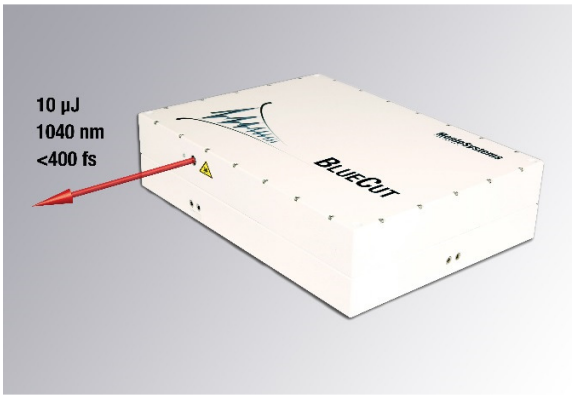

Figure 3: Menlo Systems' BlueCut microjoule femtosecond fiber laser used for photostimulation.

In collaboration with Thorlabs the Häusser group has developed a setup for *in-vivo* photoactivation of specific functional neurons and simultaneous imaging of their activity (Fig. 2). It is using **Thorlabs'** fully rotatable **Bergamo** multiphoton microscope for 2-photon imaging of brain tissue at a laser wavelength suited to the calcium sensing fluorophores, enabling the recording of >1000 neurons in one field of view. For photostimulation of the light sensitive ion channel at a different wavelength they coupled the output from **Menlo Systems' BlueCut** microjoule femtosecond fiber laser (Fig. 3) into the microscope such that the activity of the neurons can be simultaneously controlled without interfering with the optical recording.

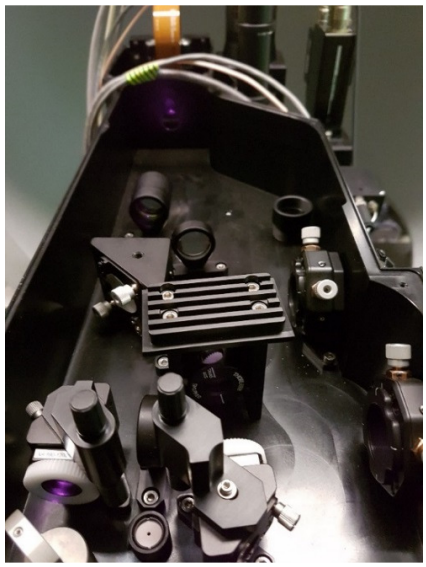

Figure 4: Stimulation light path with spatial modulator which the BlueCut laser is integrated into.

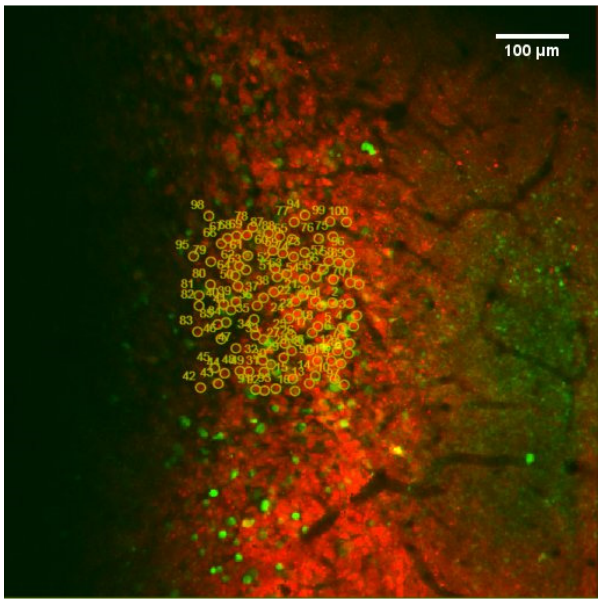

Figure 5: *In-vivo* 2-photon fluorescence image of a mouse brain. The calcium indicating protein is fluorescent in the green channel, the light sensitive ion channel is labelled with the red fluorescent mcherry protein in order to help identify the cells to be targeted by the stimulation laser. The 100 numbered circles mark target neurons.

The stimulation light path utilizes a spatial light modulator (Fig. 4) to shape the laser beam into the specific pattern required to target a certain population of neurons (Fig. 5). In this way, the neural computation can be causally probed within a brain region and the specific activity patterns of certain neurons can be linked to the behavioral output of the animal (see Fig. 1). The stimulating laser power, duration, and temporal pattern can be set to drive very well defined activity patterns in the targeted neurons.

Photons triggering action potentials during a virtual reality task

(Video available online)

With the novel photostimulation and imaging setup the UCL researchers are pioneering in the field of targeted optogenetics. Here they demonstrate the selective activation of 100 neurons in the brain of an awake and behaving mouse. The animal's running activity is coupled to movement around a virtual reality environment, allowing animals to perform complex spatial tasks while being head fixed to enable effective and accurate imaging and stimulation. In the current example the neural activity is triggered through exposure to femtosecond pulses at 1040 nm wavelength at an average power of 6 mW per cell, the duration of the exposure was 100 ms. Since the photoactivation process is based on multiphoton absorption of the NIR wavelength laser, the resulting high spatial resolution ensures the ability to address selected neurons without effecting neighboring cells. Watch the video above to see the average fluorescence response of the calcium sensitive protein to ten stimulation cycles.

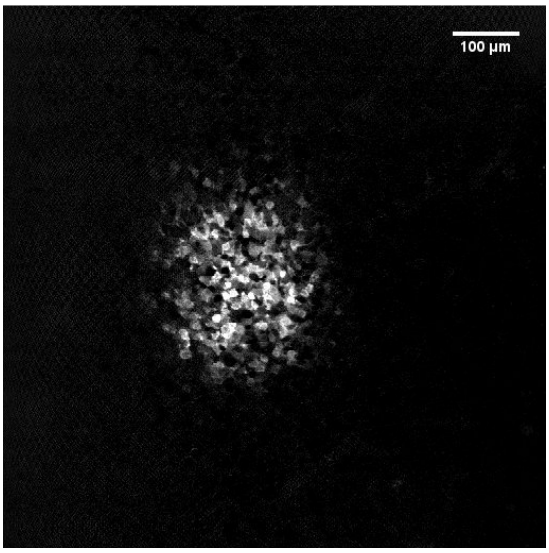

Figure 6: Fluorescence increase of brain cells 0.3s after photostimulation, average over 10 stimulation cycles.

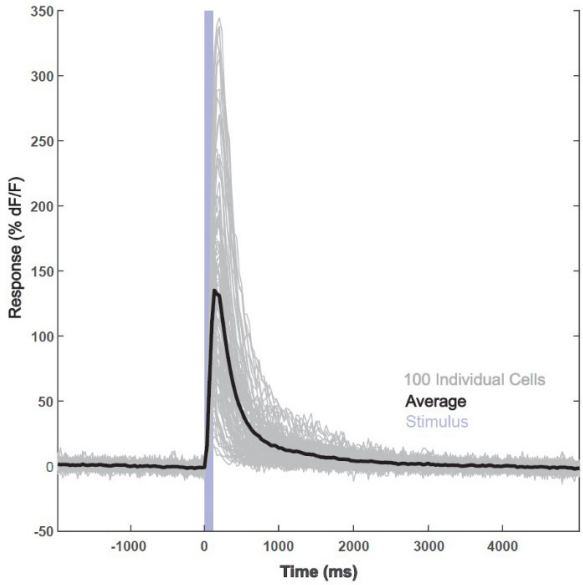

Figure 7: Temporal evolution of the fluorescence increase in 100 individual cells (grey) and the average signal (black) after photostimulation (blue bar).

In order to isolate the effect of the stimulus on the cell activity the change in fluorescence (ΔF) is divided by the fluorescence (F). The image in figure 6 captures the average $\Delta F/F$ 0.3 s after photostimulation. Figure 7 reveals the temporal progress of the fluorescence increase $\Delta F/F$ in each cell resulting from the stimulating laser pulse. On average the signal increases by more than 130 %.

The technique being developed by Prof. Häusser's group promises to enable great progress in neuroscientific research. Thorlabs and Menlo Systems feel honored to be making a contribution to such a meaningful achievement and are grateful for the opportunity to prove the excellent performance of their products.

Publications:

A. M. Packer, B. Roska, and M. Häusser: Targeting neurons and photons for optogenetics; Nature Neuroscience Vol. 16, p. 805 (2013)

V. Emiliani, A. E. Cohen, K. Deisseroth, and M. Häusser: All-optical interrogation of neural circuits; Journal of Neuroscience Vol. 35, p. 13917 (2015)

Weblinks:

Wolfson Institute for Biomedical Research:
http://www.ucl.ac.uk/wibr

Neural Computation Lab:
http://www.dendrites.org/

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