APP NOTE #23

APRIL 24

LIGHT UP THE BRAIN – TWO-PHOTON HOLOGRAPHIC OPTOGENETICS

Stimulating tens to hundreds of neurons in the brain with high spatial and temporal precision is an important topic in neuroscience today. It provides a deeper understanding of brain functions and the relation to behavior and pathological states, which is important in the research of neurological diseases. Over the last decades, various optical stimulation methods have been developed.

The most successful is two-photon holographic optogenetics which allows stimulating neurons in the brain with high precision and minimal invasion. Different stimulation processes require different amounts of pulse energy. Thus, it is important to choose the most cost-effective femto laser to ensure the correct pulse energy and laser power are available to match the stimulation scheme applied.

The aeroPULSE FS series give researchers the flexibility to choose a laser with the desired pulse energy and repetition rate required to control the right amounts of neurons for their specific application. This removes the risk of tissue damage and associated unnecessary cost, which historically existed due to the need to buy available lasers having too much excess power.

Stimulate individual neurons

Stimulating spatial and temporal patterns of brain activity lets us understand basic brain functions and their effect on general behavior and pathological states. For example, stimulations in the primary visual cortex can lead to perceptions of patterns, motions, shapes, and colors. Other studies investigate how many neurons need to be activated to generate a neural response - and in which order - or to help develop medication for, e.g., Alzheimer's disease.

To advance our understanding of the neural activity, we need to enable stimulation of individual neurons, either simultaneously or following specific temporal patterns. The subsequent neural activity is then monitored near the stimulation site or, ideally, throughout different brain regions.

Traditionally, scientists probed the brain with electrodes to stimulate and record activity, but these approaches did not allow for specific targeting and are intrinsically invasive. To overcome these challenges, two-photon holographic optogenetics was introduced more than ten years ago, which has significantly developed to become a standard tool for present-day neuroscience research.

In two-photon holographic optogenetics, ultrashort femtosecond light pulses are used for patterned illumination in the brain to activate or suppress the activity of neuronal ensembles or both.

The advantages over other techniques are that neuroscientists can make deeper stimulations in highly scattering brain tissue and specifically target up to hundreds of neurons with sub-cellular resolution and high temporal precision.

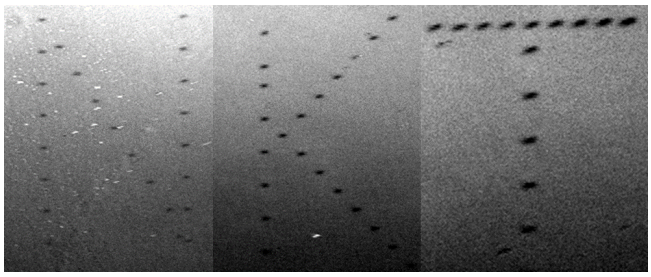

Figure 1: Example of holographic pattern generated for three-dimensional scan less holographic optogenetics with temporal focusing (3D-SHOT) using an aeroPULSE FS50 with a Meadowlark Spatial Light Modulator (SLM). Courtesy of Lamiae Abdeladim and Hillel Adesnik, UC Berkeley.

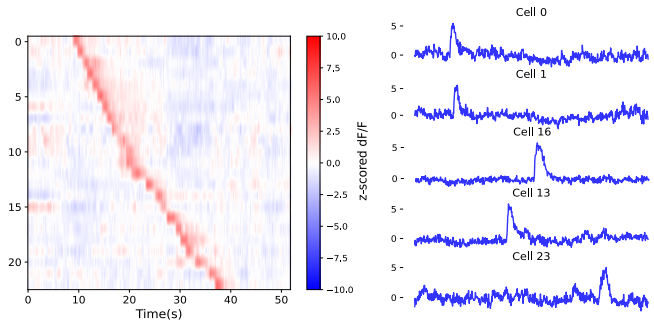

Figure 2: Sequential single-neuron stimulation at P=25 mW/30x15 ms pulses at 30 Hz using an aeroPULSE FS50. Shown on the left is a change in the fluorescent signal from a total of 22 neurons. Individual traces are shown on the right. Courtesy of Lamiae Abdeladim and Hillel Adesnik, UC Berkeley.

In optogenetics, light induces neural activity in defined cell types expressing photo-sensitive microbial opsins, that either generate or suppress neuronal activity.

When near-infrared light is used, e.g., 1030 nm femtosecond pulses, in a two-photon process for opsin activation, the neuroscientist can control the stimulation in highly scattering brain tissue.

Finally, a spatial light modulator (SLM) coupled with computer-generated holography (CGH) can generate holographic three-dimensional light patterns (see Figure 1) to specifically target individual neurons within a genetic subtype.

This approach has the advantage of arbitrarily controlling the spatial distribution of light in the brain and selectively activating individual neurons with remarkable spatiotemporal precision (see Figure 2).

Flexible pulse energies and repetition rates

Higher pulse energies are required to stimulate tens to hundreds of neurons in the brain using two-photon optogenetics. To avoid tissue damage during stimulation, repetition rates of hundreds of kilohertz up to 10 MHz are applied.

On the other hand, the commercialization of ultrafast fiber lasers over the last decade has given access to pulse energies of several microjoules and repetition rates tunable up to tens of MHz at the 1-micron wavelength. With a pulse duration of several hundred femtoseconds, fiber lasers are the ideal light source for photostimulation.

New probing methods for photostimulation were developed using ultrafast fiber lasers together with SLMs. Spiral scanning and temporal focusing modalities are amongst the most popular techniques.

- In spiral scanning, the soma is scanned in a spiral pattern to generate a neural response.
- In temporal focusing, to avoid degradation of axial resolution in an expanded beam, the light is chirped before the focal spot, where it recombines temporally, allowing to reinstate axial resolution.

The two methods require different pulse energies. Typically, a pulse energy of a few microjoules is needed for spiral scanning. For temporal scanning, up to tens of microjoules can be required.

With the aeroPULSE FS series, the neuroscientist can obtain the desired optical performance suited for spiral or temporal techniques without having to budget for an over-priced, over-specified laser.

aeroPulse FS50: With >50 W average power and >40 µJ pulse energy, the FS50 is the perfect solution for temporal focusing modalities as well as splitting one laser between two microscopes.

aeroPulse FS10: With >10 W average power and >20 µJ pulse energy, the FS10 is ideal for spiral scanning modalities. The FS10 has the added advantage of being air-cooled.

Opsin stimulation & the role of pulse width

In contrast to two-photon imaging, the pulse duration, and the equivalent peak power, play a less significant role in two-photon stimulation of opsins generating a neural response. Opsins, with relatively long excited-states lifetimes, can be stimulated with pulse durations between 270 fs and 400 fs and give a similar number of activated cells (see Figure 3).

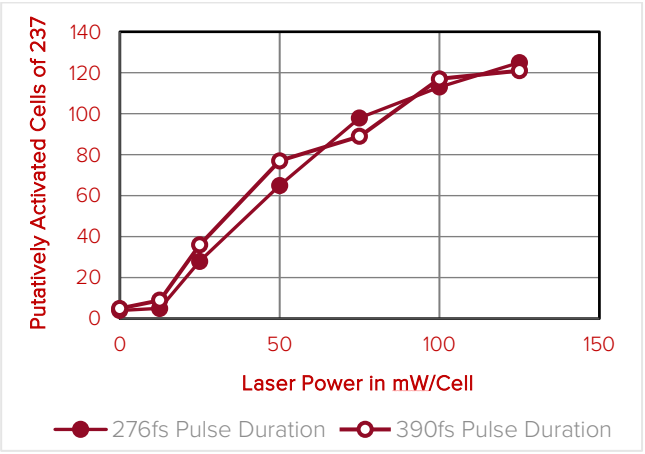

Figure 3: "Stim Test" using two pulse durations of 276 fs and 390 fs showing the number of activated cells vs laser input power. No significant difference in stimulation between the two pulse durations was detected in these experiments. Courtesy of Ian Oldenburg, Rutgers University.

The FS10 and FS50 have a typical pulse duration of around 270-400 fs, making the products ideal for photostimulation applications.

High reliability and uptime

Reliability and uptime are key in optogenetics, especially when lasers are deployed in animal studies. All aeroPULSE FS products are based on our proprietary aeroGAIN PCF fiber amplifier technology.

This technology is well-established with over 10,000 ultrafast lasers deployed with this platform worldwide in medical, industrial, and academic applications. With this peace of mind, one can be assured of the high reliability and uptime of the aeroPULSE FS lasers, which is backed up with an attractive warranty package.

Summary

In two-photon holographic optogenetics, the availability of femtosecond optical power with high reliability is essential. The aeroPULSE FS laser portfolio provides neuroscientists with flexible choices of power and with peace of mind that the laser can be relied upon to perform on demand making the aeroPULSE FS laser an essential photostimulation tool in any optogenetics lab!

WHY AN AEROPULSE LASER?

- High uptime
- Choose from two different models depending on your power needs
- The most cost-effective laser on the market
- Designed and manufactured to provide stable & reliable performance
- Backed by a 2-year warranty