Silvia Dante, Marco Salerno, Emanuele Marconi, Luca Berdondini.

Neuronal networks produced in cell culture are a unique tool to provide information about synapses formation, development and functionality. Several geometrical, physical and chemical cues play a role in neuron adhesion and neurite outgrowth. We have used a nano-drop method to pattern agarose coated surfaces with an adhesion protein (PDL) and we have subsequently monitored the growth of neural networks. Depending on its thickness, the intermixed agarose/PDL layer was proved to be a successful substrate to confine the neural network and to guide its growth. In particular, for thickness of the polymer cushion below 100 nm, neurons formed connections and remained in morphologically healthy conditions up to 21 DIV. The correlation between surface properties (morphology, stiffness, roughness, charge) and neuron adhesion and survival was investigated by AFM. Force-Volume mapping of the surface showed a dominating effect of surface stiffness vs topography. In order to allow connectivity, a critical distance of 80 μm between spots of adhesion protein was found. Finally, the functionality at 21 DIV of 2D networks grown on agarose/PDL substrates patterned by micro contact printing was proved by patch clamp and by the measurements of the basic activity on micro electrode arrays.

Deep Brain Optogenetic Stimulation Using Bessel Beam

Aswini Kanneganti, Shivaranjani Shivalingaiah, Ling Gu, George Alexandrakis, Samarendra Mohanty.

Optogenetics is emerging as a method to stimulate and probe in-vivo neural circuits with high cellular specificity achieved by genetic targeting; and precise temporal resolution provided by interaction of light-gated ion-channel Channelrhodopsin-2 (ChR2) with blue stimulation beam. Since the biological tissue exhibits higher attenuation due to absorption and scattering in the blue activation spectrum, it leads to lowering of the intensity and spatial resolution as the light travels to further depths. Further, diverging blue light emanating from optical fiber or LED, used for in-vivo stimulation, requires placement of the light source near the stimulation region implying deep surgical implantation. While we demonstrated higher resolution and depth of stimulation by two-photon microbeam, effective in-vivo activation of targeted cells over large area necessitates use of single-photon beam. In order to achieve deep-brain optogenetic stimulation by single-photon, here we propose use of non-diffracting Bessel beam instead of Gaussian beam. For generating and delivering the Bessel beam via optical fiber, a micro-axicon was fabricated at the tip. Free-space propagation of Gaussian beam from the cleaved fiber was compared to that of the Bessel beam. The large propagation distance, characteristics of Bessel beam is better suited for in-depth single as well as two-photon optogenetic stimulation of the ChR2 sensitized cells. To validate this in neuronal tissue, theoretical simulations were conducted using Bessel and Gaussian beams based on Monte Carlo photon transport method applied on layered mouse brain geometry. In contrast to Bessel beam, Gaussian beam due to its divergence coupled with the inherent tissue optical properties was found to travel very limited depth in the tissue and the intensity below few mW/mm2 not being sufficient to stimulate the ChR2 sensitized cells. We will present results of both theoretical simulations and validation experiments.

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