Tether-less Implantable Upconverting Microscale Light Bulbs for Deep Brain Neural Stimulation and Imaging

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Abstract: We demonstrate the design and fabrication of implantable micro-scale light "bulbs" comprising of parylene-encapsulated upconverting lanthanide-doped nanocrystals (absorbing near-infrared and emitting blue light) for non-invasive targeted optogenetic stimulation of local neuronal ensembles.

OCIS codes: (190.7220) upconversion; (170.4090) Modulation techniques; (170.0170) Medical devices and biotechnology; (160.4330) Nonlinear optical materials

1. Introduction

One of the fundamental difficulties in neuroscience experiments is the lack of tools for chronically monitoring and modulating the activity of local neuronal ensembles simultaneously in different regions of the brain. Despite recent advances in neural modulation techniques, including a rapidly expanding optogenetic toolset [1, 2], we still lack a robust, minimally-invasive optogenetic stimulation platform. The ability to independently deliver light to multiple, highly localized (~25 μm^3) regions of the brain would drastically improve in vivo optogenetic experiments. A major challenge is delivering light to a specific subset of cells that have expressed opsins. Illuminating a large volume of brain using light sources above the brain surface does not provide the requisite spatial resolution and since the intensity falls off rapidly, only a small fraction of target neurons in the vicinity of the light source (~200 μm) will be excited. Increasing the light source power, on the other hand, results in the generation of excessive heat in the brain and the potential for phototoxicity. Using the Kubelka–Munk model [3] we can calculate the intensity drop as light undergoes scattering, absorption, and Gaussian diffraction spreading in the tissue and its intensity rapidly falls below the excitation threshold of opsins. Given a scattering coefficient of 11 mm^{-1} in the mouse brain and the threshold intensity of 1 mW/mm^2 for a channelrhodopsin to evoke action potentials, an input power of 2.25 mW is required in a fiber optic (200μm, NA=0.37) to excite a neuron at a depth of 2 mm into the cortex, resulting in a (very high) intensity of 71.6 mW/mm^2 at the output aperture of the fiber; this is sufficient to cause damage to the brain tissue. This trade-off between the range of stimulation and the required optical power results in an inherently low spatial resolution. The absorption of light in the brain tissue at the optogenetic wavelengths for stimulation and inhibition of neuronal activity (470 nm and 590 nm, respectively) is high. To reach deeper brain regions, bare fibers (usually 20-200μm in diameter) are inserted, causing a large displacement of tissue and vasculature and producing a tethering force on the brain tissue. The absorption coefficient in the visible range is at least 10 times larger than the absorption of light in the NIR range from about 750 nm to1200 nm (the optical window). To alleviate this problem, here we demonstrate micro-pillars (Fig. 1a) realized in parylene C with embedded blue-emitting lantanide-doped upconverting nanocrystals (UCNPs) can be excited with NIR pump (at λ=980 nm) to perform optogenetic stimulation of local neuronal assemblies.

2. Excitation of UCNPs Through a Brain Slice Tissue

Building on our previously reported lanthanide-doped upconverting nanocrystals [4], we have optimized the doping concentrations to achieve three-photon absorption and subsequent blue-shifted emission at visible wavelengths. Specifically, by optimizing Tm and Yb dopants at concentrations of ~2% and 40%, respectively, we could realize 10-nm doped $NaYF_4$ nanocrystals that absorb NIR at the wavelength of λ = 980 nm and emit light in emission bands centered on λ = 800 nm and λ = 451 nm (Fig. 1b). Our UCNPs demonstrating three-photon upconversion are orders of magnitude brighter than organics and comparable to conventional two-photon UCNPs, despite the lower-energy excitation and near-infrared emission. To demonstrate the efficacy of these nanocrystals for deep brain optogenetic stimulation, we prepared coronal brain slices from a wild-type mouse brain with different thicknesses ranging from 150 μm to 500 μm as shown in Fig. 1c. To perform a proof of concept imaging experiment, we coated the surface of a glass cover slip with our nanocrystals and then sandwiched the brain slice in between the cover slip and a 1 mm thick glass slide as shown schematically in Fig. 1d.

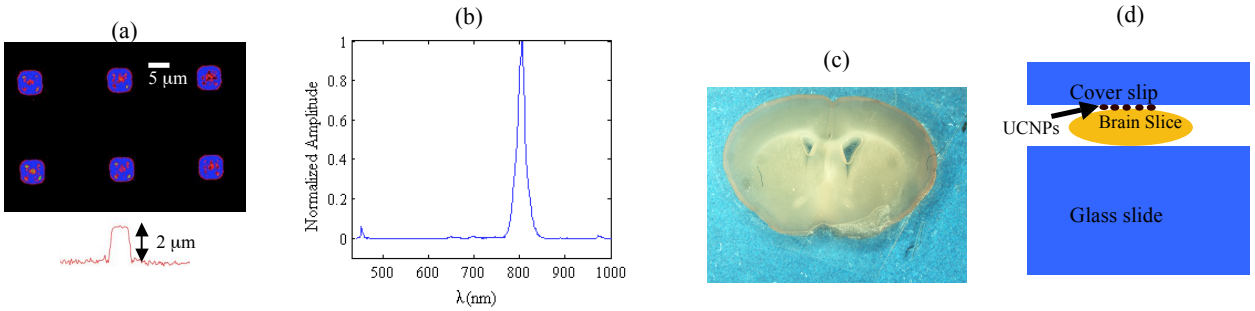

Fig. 1 (a) 3D confocal microscopy image of parylene C pillars encapsulating UCNPs. (b) The normalized spectrum of the UCNP emission, showing two emission bands centered on λ = 800 nm and 451 nm. (c) A 500 µm slice from a mouse brain. (d) The sandwich arrangement, where brain slice is sandwiched between a glass slide and a cover slip.

We pumped the nanocrystals on the surface of the brain slices and also through the whole thickness of the brain slices by flipping the sample in our inverted confocal microspectroscopy setup schematically shown in Fig. 2a. The emission spectra of the nanocrystals on the brain slice surface and through the slice for a slice thickness of 150 µm is shown in Fig. 2b and Fig. 2c, respectively. As it can be seen from Fig. 2c, the blue emission band is completely absorbed through the tissue and the 800 nm emission band has passed through the tissue, which clearly indicates the possibility of excitation of nanocrystals through the whole thickness of the tissue.

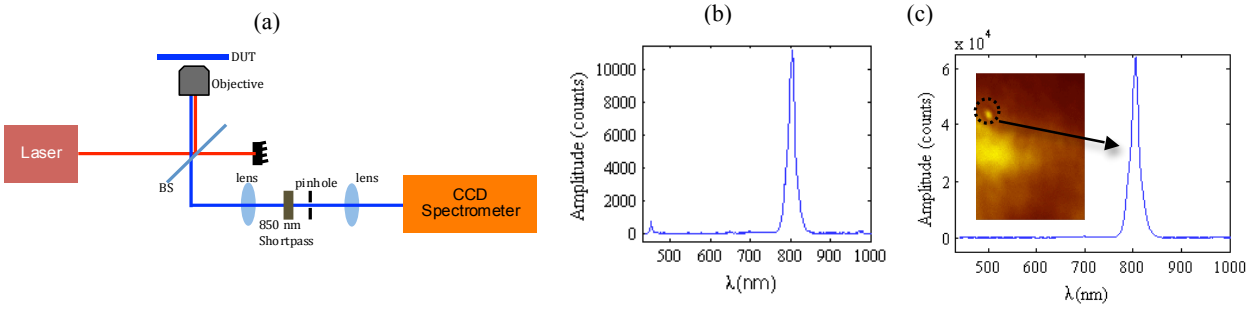

Fig. 2 (a) 3D confocal microscopy image of parylene C pillars encapsulating UCNPs. (b) The normalized spectrum of the UCNP emission, showing two emission bands centered on λ = 800 nm and λ = 451 nm. (c) A 150 µm slice from a mouse brain. (d) The sandwich arrangement in which a brain slice is sandwiched between a glass slide and a cover slip.

The higher photon count through the brain slice tissue is because of the higher concentration of the UCNPs on the recording spot shown in the inset of Fig. 2c as the integrated emission image obtained through raster scanning the sample. We used a pump power of 630 µW on the sample and an integration time of 10 sec. To investigate whether the local blue emission of the nanocrystal clusters is enough for optogenetic stimulation of channelrhodopsins, we estimated the peak emission intensity at λ=451 nm from our spectroscopy measurements. It appears that with a reasonably large density of particles, we can achieve an intensity of 0.3 mW/mm^2 on the UCNPs clustered in microscale light bulbs, which is close to the threshold of excitation of channelrhodopsins. We will discuss further details of our optogenetic experiments in an upright electrophysiology rig using the patch-clamp technique in the presentation.

3. References

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