SUPPLEMENTARY INFORMATION

Fast targeted gene transfection and optogenetic modification of single neurons using femtosecond laser irradiation

Maciej Antkowiak,[1,2,4*] Maria Leilani Torres-Mapa,[2] Emily C. Witts,[3] Gareth B. Miles,[3] Kishan Dholakia,[2] Frank J. Gunn-Moore[1]

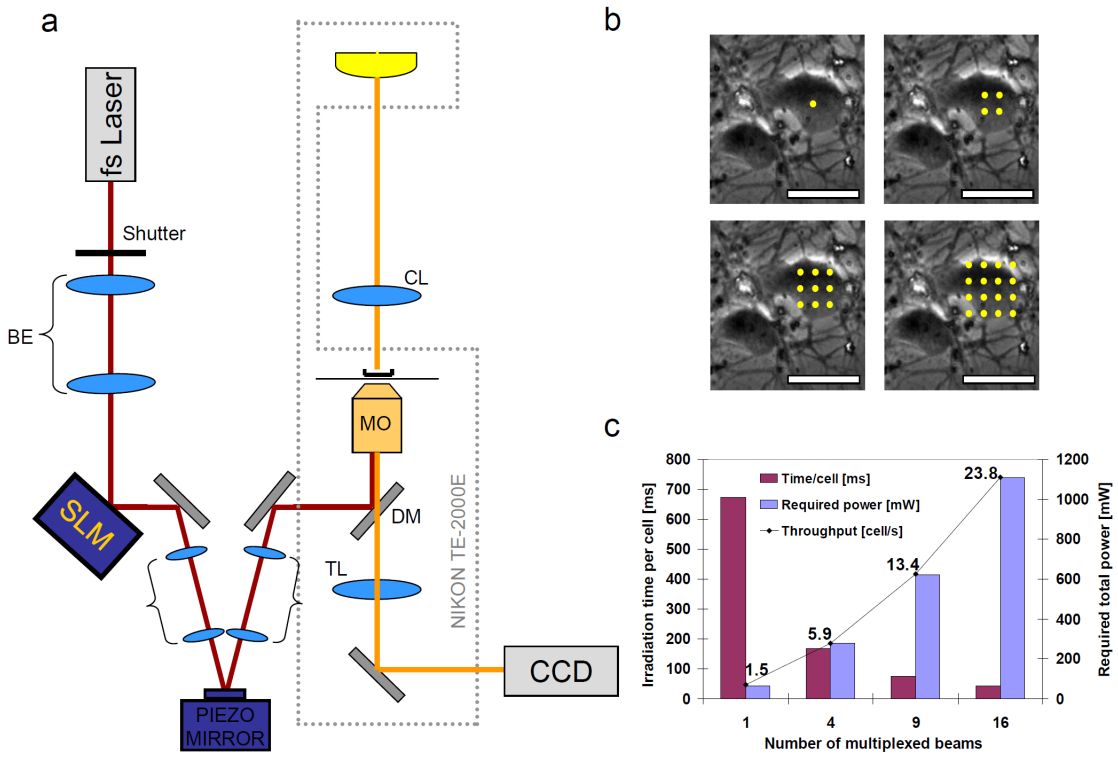
[1] University of St Andrews, School of Biology, St Andrews, UK

[2] SUPA, University of St Andrews, School of Physics & Astronomy, St Andrews, UK

[3] University of St Andrews, School of Psychology and Neuroscience, St Andrews, UK

[4] SULSA, Scottish Universities Life Sciences Alliance

[*]Correspondence: ma81@st-andrews.ac.uk (M.A.)



Supplementary Figure S1. SLM-based femtosecond optical transfection system. (a) Schematic of the experimental setup. SLM – Spatial Light Modulator, BE: Beam Expander (8x); CL: microscope condenser lens; MO: microscope objective; DM: NIR dichroic mirror; TL: tube lens; CCD: digital camera. (b) The phase mask displayed on the SLM can create spatially multiplexed beams enabling simultaneous irradiation of multiple sites on a cell's membrane. Scale bars in DIC images are 20 µm. (c) The increasing number of multiplexed beams increases the demand on total power of the laser beam at the back aperture of the microscope objective (right axis) but also significantly reduces the time required to irradiate a given total number (16 in this example) of sites on cell's membrane (left axis). This significantly improves the achievable throughput of transfection (numbers above black data points, [cells/second]). the variation in irradiation time and total power is negligible and these values can be treated as constant for any

given irradiation conditions. The throughput is calculated based on the irradiation time per cell and does not account for any delay related to the manual selection of the irradiated cells.

Supplementary Video 1. The touch-screen interface enables rapid “point-and-transfect” targeting of selected cells upon a touch of a finger by combining the use of a fast steering mirror and user-friendly software (LabView). This example video shows in real time (25fps) a sequential irradiation of 16 spots on each targeted cell with a 40 ms dose (as discussed in the text) guided by a DIC image. The scale bar corresponds with 50 μm in the microscopy image and 4.1cm in the real dimensions of the touchscreen.