Suspended-drop electroporation for high-throughput delivery of biomolecules into cells

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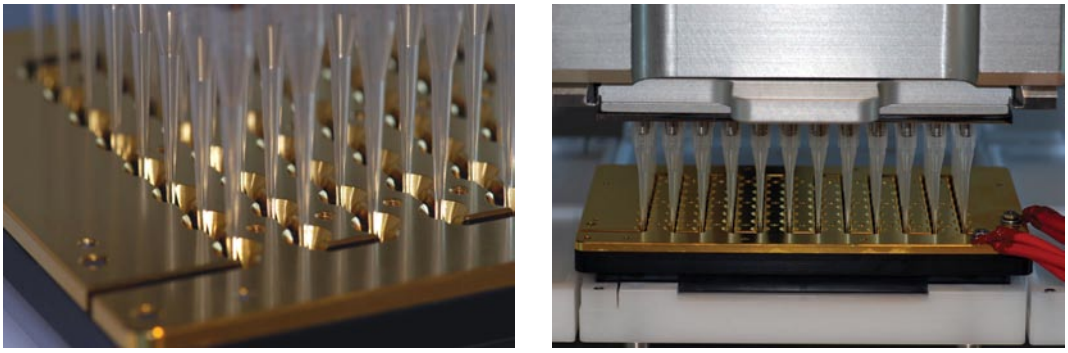
Supplementary figures and text:

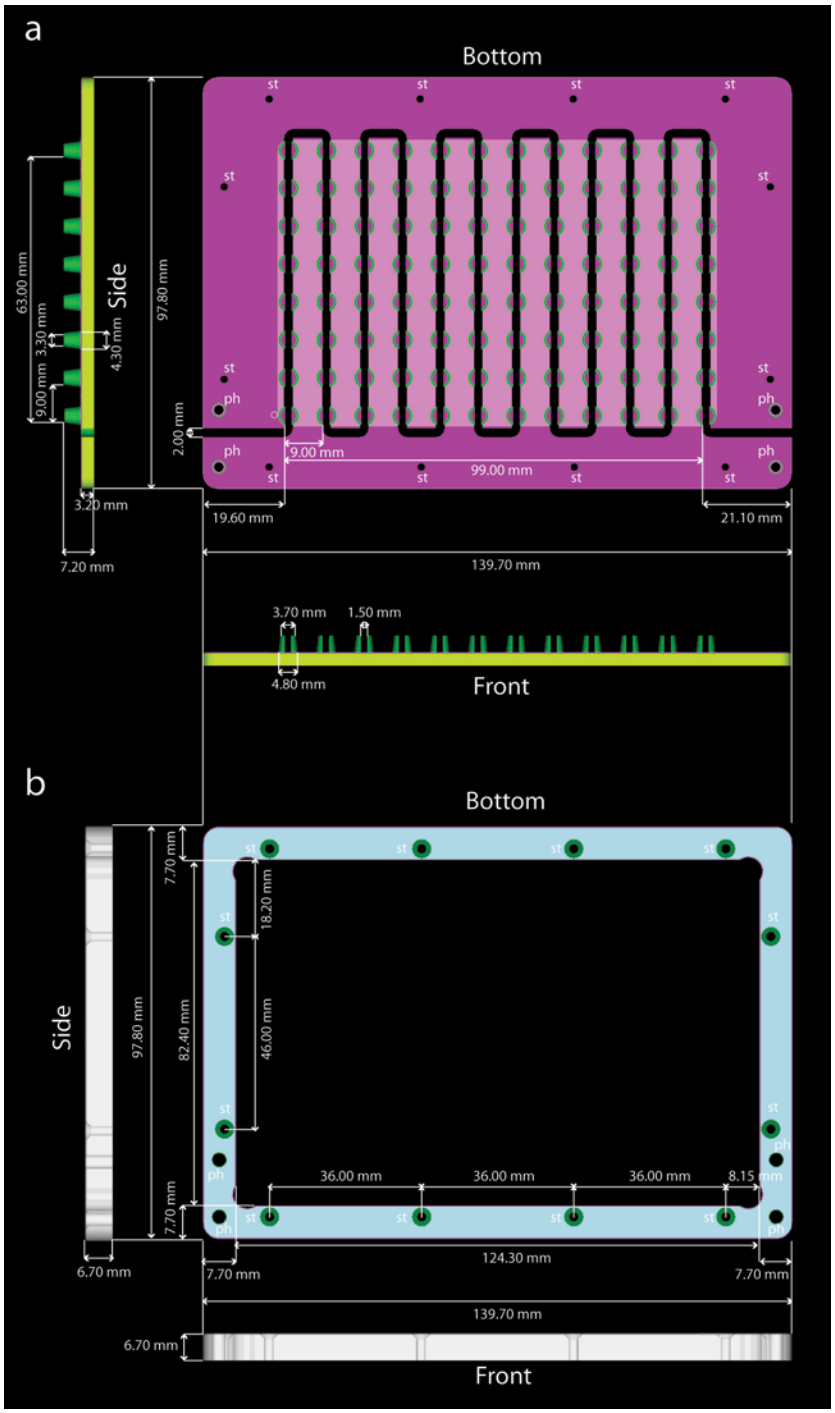
Supplementary Figure 1



Supplementary Figure 1: The SDE-device combined with a 96-channel pipetting robot (Apricot Technology).

The 96 electroporation chambers are simultaneously loaded, samples are electroporated and ejected, in less than 1 minute.

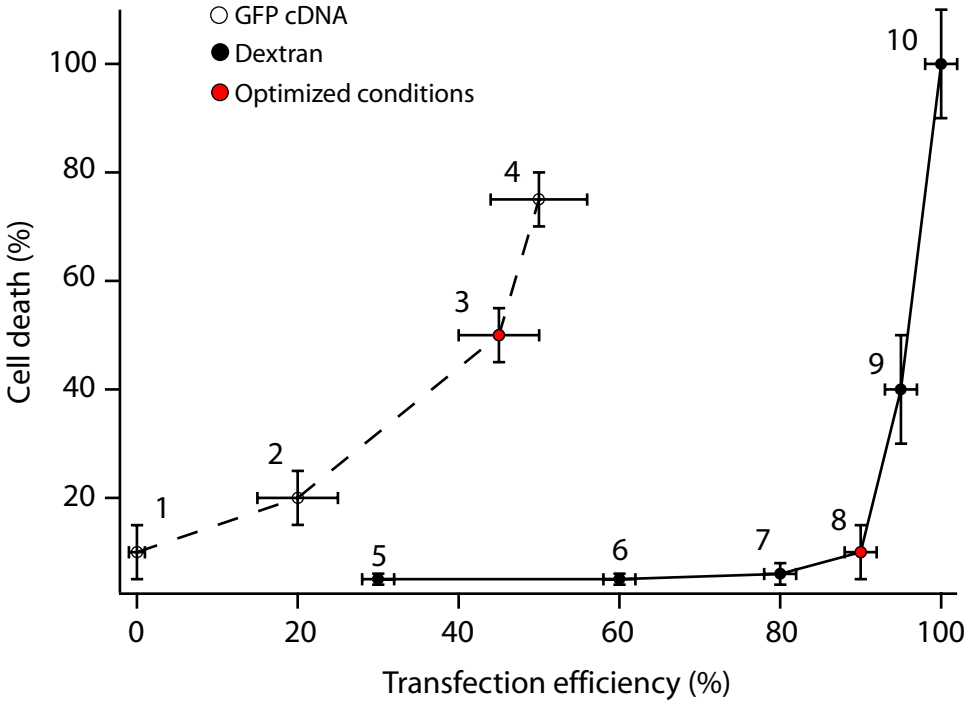
Supplementary Figure 2



Supplementary Figure 2: Construction plan of the SDE-device.

(**a**) Bottom, side and front view of the two juxtaposed gold-coated copper pieces. The two copper pieces are assembled onto a delrin frame (**b**) with 12 screws (st: screw tap) and four pins (ph: pin hole). The different components of the SDE-device were machined with a precision of ± 0.02 mm.

Supplementary Figure 3

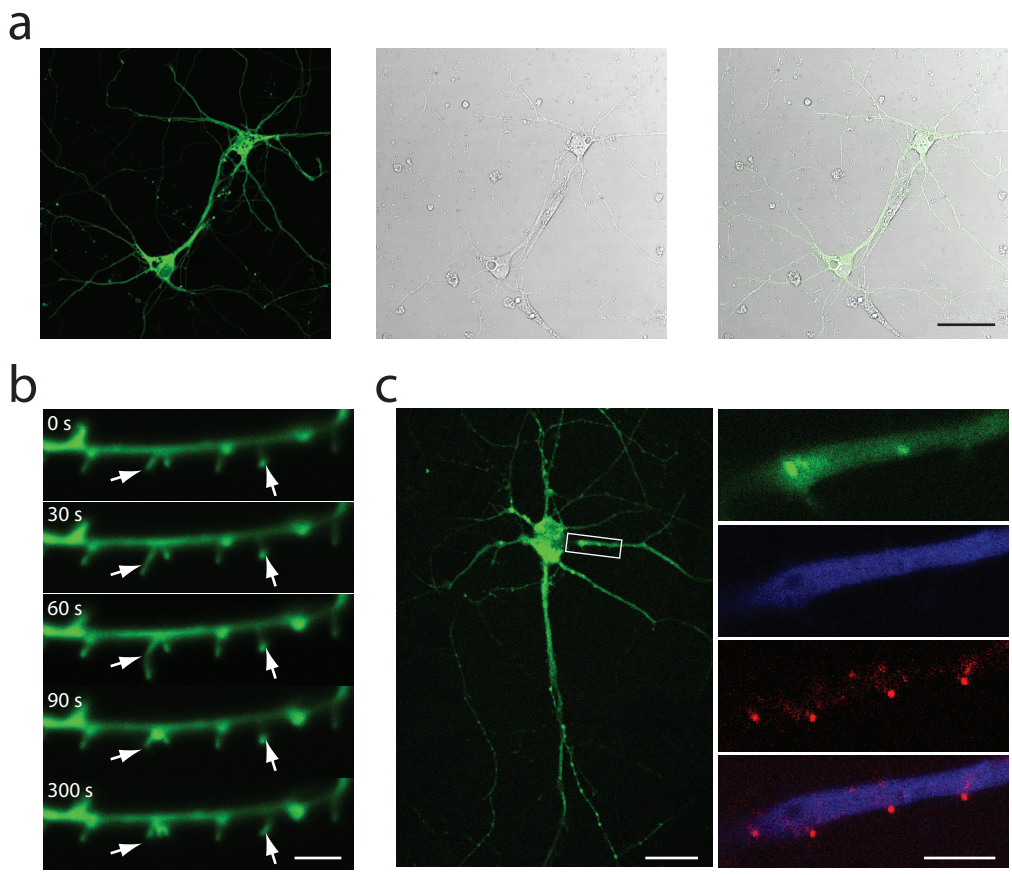


Conditions	Pulse Intensity (V/cm)	Pulse length (ms)	Number of pulses
1	400	20	1
2	500	20	1
3	670	20	1
4	860	20	1
5	400	7	2
6	470	7	2
7	530	7	2
8	630	7	2
9	730	7	2
10	870	7	2

Supplementary Figure 3: Correlation between cell death and transfection efficiency.

Cell death percentage versus transfection efficiency of differentiated HL-60 cells electroporated with GFP cDNA (dashed line) and 10kDa fluorescein-labelled dextran (continuous line) for increased voltage settings. The table presents the pulse settings for each point in the graph. Optimized conditions are highlighted in red (see **Supplementary Table 2**).

Supplementary Figure 4



Supplementary Figure 4: Electroporated neurons show morphological normal development.

(**a**) Differential interference contract image of rat primary hippocampal neuron 8 days after electroporation of GFP cDNA. (**b**) Electroporated GFP-expressing hippocampal neurons at DIV8 show filopodia dynamics. Arrows highlight a dynamic dendritic filopodia (left arrow) that extend transiently and a spine (immobile protrusion, right arrow) (Ziv and Smith, 1996). (**c**) Fixed GFP-expressing neurons at day 8 show local GLUR2 staining characteristic of nascent synapses (GLUR2 shown in red and dendritic MAP2-staining shown in blue). Scale bars, 50µm in **a**, 5 µm in **b**, 20 µm in **c** left and 5 µm in **c** right.

Supplementary Table 1. Comparison of the delivery efficiency of cDNA, siRNA and dextran into differentiated HL-60 cells with different electroporation buffers and devices.

While cDNA transfection efficiency was similar between the SDE-device and the Amaxa system, the SDE-device had better performance compared to the Amaxa system in terms of Dextran delivery as well as in cell viability when delivering siRNAs. Note that the Amaxa system generates significantly more observable cell debris as well as cell fusion compared to the SDE device. The SDE-device also had a significantly better characteristics for the delivery of cDNA and siRNA when compared to the Ambion system.

	Amaxa 96-well shuttle (Nucleofector buffer)		Ambion siPorter 96 well electopration (siPort buffer)		SDE-device (Extracellular buffer)	
	Transfection efficiency (%)	Cell death (%)	Transfection efficiency (%)	Cell death (%)	Transfection efficiency (%)	Cell death (%)
cDNA†	50	45	1-2	50	45	40-45
siRNA††	90-95	50	-	-	90-95	5-10
Dextran†††	0	50	80	10-20	90	5-10
Buffer cost (1litter)	~120,000$		~8,000$		~2$	

†GFP cDNA, †† Lamin A/C siRNA, ††† 10 kDa fluorescein-dextran.

Supplementary Methods

Electroporation buffer. The electroporation buffer used was 5mM KCl, 125 mM NaCl, 1.5 mM $CaCl_2$, 1.5 mM $MgCl_2$, 10 mM D-glucose and 20 mM HEPES; pH was adjusted to 7.4. The solution was filtered and kept at 4°C for up to two months. All chemicals were purchased from Sigma at the highest available purity.

HL-60 cell culture and differentiation. HL-60 cells (ATCC, cat. CCL-240) were cultured as recommended by the provider in Iscove's Modified Dulbecco's Medium (Invitrogen, cat. 12440) supplemented with 20% fetal bovine serum (Invitrogen, cat. 26140) and penicillin-streptomycin-glutamine (Invitrogen, cat. 10278) under humidified 5% CO_2 atmosphere at 37 °C. Cells were differentiated with 1.3% DMSO (Fluka, cat. D2640) in complete growth media and cultured for 3-5 days.

Electroporation of fluorescein-labeled dextran. 2 million differentiated HL-60 cells were harvested and resuspended in 2 ml extracellular buffer containing 50 μM fluorescein-labeled 10 kDa dextran (Invitrogen, cat. D-1821). 20 μl of resuspended cells were loaded to each chamber of the SDE device and electroprated (**Supplementary Table 2**). Two different procedures were used for the quantification of cell death and transfection efficiency:

(i) For the quantification of cell death (**Fig. 1c**), electroporated samples were ejected from the SDE device with 150 μl complete gowth media, spined down and resuspended into fresh complete gowth media containing 500 ng/ml propidium iodide (Invitrogen, cat. P-3566) (stain nucleus of disrupted plasma membrane cells) and 500 nM ER-tracker blue-white (Invitrogen, cat. E-12353) (stain the ER of living cells). Cells were plated on fibronectin-coated Lab-Tek chambers (8 wells) and imaged (**Fig. 1c**); no washing is performed so that dead cells can also be counted.

(ii) For the quantification of transfection efficiency (**Fig. 1d**) as well as for the comparison of delivery efficiency between wells (**Fig. 1d**), each electroporation chamber was ejected with 150 μl complete growth medium and cells were plated in a fibronectin-coated 96-well clear bottom costar plate (Corning Inc., cat. 3904). Cells adhering to the

fibronectin-coated plate were washed, stained with Hoechst (650 ng/ml, for 30 minutes) (Invitrogen, cat. 33342) and imaged. Fluorescent intensity quantification was performed for more than 2000 cells in each well using the ImageXpress Consol (Molecular Devices) and using Hoechst staining as a mask. Fluorescent background of HL-60 cells (**Fig. 1d**, blue line) was determined in the same way except that the cell- fluorescein- dextran mixture was not electroporated before being ejected.

Electroporation of HL-60 cells with siRNAs. Non-differentiated HL-60 cells were harvested and resuspended in extracellular buffer to a concentration of 2 x 10^6 cells/ml. For each well, 20 µl of resuspended cells were mixed with 0.75 µl of 20 µM siRNA (Lamin A/C siRNA, cat. M-004978; siControl non-targeting siRNA, cat. D-001810-01; both from Dharmacon) and loaded into the SDE device. After electroporation (see **Supplementary Table 2**), cells were ejected with 150 µl complete growth media supplemented with 1.3% DMSO and cultured.

Western blot and immunostaining. Knockdown efficiency in siRNA experiments was determined by Western blot and immunostaining 3 days after electroporation. Cells were harvested and lysed in 60 µl of sample buffer. 10 µg of total protein amount (~20 µl of sample) was loaded and run on a 10% polyacrylamide gel. All other steps were performed using standard protocols. Nitrocellulose membrane was stained using anti-Lamin A/C and actin antibodies, and imaged (Odyssey, Li-Cor Bio.). The following primary and secondary antibodies were used: Lamin A/C rabbit polyclonal IgG (Santa Cruz Biotechnology, cat. sc-20681), actin mouse monoclonal IgG (MAB1501, Chemicon), goat anti-mouse IRDye 800CW (Li-Cor Bio., cat. 827-08364) and goat anti-rabbit IRDye 680 (Li-Cor Bio., cat. 827-08367).

For immunostaining, cells were transferred into a fibronectin-coated 96 well costar plate. 30 minutes after plating, cells were washed with PBS (Invitrogen, cat. 10010), fixed 5 minutes with ice-cold methanol, and permeabilized with 0.2% Triton X-100 (Sigma, cat. 112K09721) for 15 minutes. Lamin was stained with Lamin A/C rabbit polyclonal IgG and Alexa Fluor 594 goat anti-rabbit IgG (Invitrogen, cat. n° A11037) as primary and secondary antibodies, respectively. Nuclei were stained with Hoechst (650

ng/ml). More than 1,000 cells were imaged in each well and nuclear fluorescence intensities were quantified using Hoechst stain as a mask. Fluorescent intensities quantification was performed using the ImageXpress Consol (Molecular Devices).

Electroporation of cDNA into differencitated HL-60 cells. For a single well electroporation, 20,000 differentiated HL-60 cells were harvested and resuspended into 20 µl extracellular buffer and 0.5 µg pmaxGFP (Amaxa). The sample was loaded into a chamber of the SDE device, electroporated (see **Supplementary Table 2**), ejected with 150 µl complete growth media, and cultured. 7 hours after treatment, cells were transferred into a 8 well fibronectin-coated Lab-Tek chamber, washed and imaged.

Under-agarose chemotaxis assay. Differentiated HL-60 cells, electroporated with pmaxGFP 7 hours previous to experiments, were plated in a 96-well fibronectin coated costar plate. The under agarose assay was processed as described in Heit *et al.* with a chemoattractant source of 50 nM formyl-methionine-leucine-phenylalanine (fMLP) (Sigma, cat. F-3506) in the center of the well.

Hippocampal neurons: Culture and electroporation. Rat E18 hippocampus, in hibernated buffer E/B27, were purchased from Brainbits (Springfield, IL, USA). Hippocampi (~10^6 neurons) were disrupted by gently pipetting, harvested by centrifugation, and resuspended into 100 µl electroporation buffer with 1 µg pmaxGFP (Amaxa). 20 µl/chamber were loaded in the SDE device and electroporated (see **Supplementary Table 2**). Cells were washed with 150 µl neuron growth media and plated into two wells of a four well poly-lysine coated Lab-Tek chambers. Cells were cultured under humidified 5% CO_2 atmosphere at 37 °C. Every three days, 200 µl media was removed and replaced with 300 µl fresh media.
Neuron plating growth media contained 89 ml minimum essential media without glutamine and phenol red (Invitrogen, cat. 51200), 5 ml fetal bovin serum (Invitrogen), 2 ml 1 M D-glucose (Sigma, cat. G7021), 2 ml 50x B-27 supplement (Invitrogen, cat. 0247), 0.5 ml 100 mM sodium pyruvate (Invitrogen, cat. 11360), 1 ml 1 M HEPES buffer solution (Invitrogen, cat. 03711), 0.25 ml penicillin-streptomycin-glutamine (Invitrogen,

cat. 10278) and 100 µl serum extender (Becton D., cat. 355006). Growth media was kept at 4°C for 5 days.

For immunostaining, hippocampal neurons were fixed with 4% PFA, permeabilized with 0.2% Triton X-100 (Sigma, cat. 112K09721) for 15 minutes. Map2 and GluR2 were stained using rabbit anti-MAP2 (Chemicon, cat. AB5622) mouse anti-GluR2 (BD Bioscience Pharminger, cat. 556341) as primary antibodies. Alexa Fluor 594 goat anti-mouse IgG (Invitrogen, cat. A-11032) and Alexa Fluor goat anti-rabbit IgG (Invitrogen, cat. A-21068) were used as secondary antibodies.

3T3-L1 adipocytes: Culture and electroporation. 3T3-L1 cells (ATCC, cat CL-173) were cultured and differentiated as recommended by the provider (see also Tengholm et al.) under humidified 10% CO_2 atmosphere at 37°C. For a single well electroporation, 20,000 cells were harvested and resuspended in 20 µl extracellular buffer with 0.5 µg pmaxGFP. The sample was loaded into the SDE device, electroporated (see **Supplementary Table 2**) and ejected with 150 µl complete growth media into a poly-lysine coated Lab-Tak chamber. Cells were imaged one day after treatment.

RBL-2H3 tumor mast cells: Culture and electroporation. RBL-2H3 cells (ATCC, cat. CRL-2256) were cultured as recommended by the provider under humidified 10% CO_2 atmosphere at 37°C. Cells were treated and imaged as described for 3T3-L1 cells.

HUVEC: Culture and electroporation. HUVEC cells (ATCC, cat. CRL-2873) were cultured as recommended by the provider under humidified 10% CO_2 atmosphere at 37°C. Cells were treated and imaged as described for 3T3-L1 cells.

Fluorescence microscopy. 96-well plates were imaged with an ImageXpress 5000A (Molecular Devices). Lab-Tek chambers were imaged using a LSM 5 Pascal confocal microscope (Zeiss) equipped with a 20X, 40X oil immersion and 63X water immersion objectives. Corresponding excitation and emission filters were used for the different fluorescent compounds.

Power supply. Three 44,000 µF 100Vdc capacitors are wired in series to achieve a maximum capacity of 14,667uF @ 300Vdc. Typically the capacitor are charged by a custom built power supply to the desired DC voltage and then discharged across the electrodes by means of a solid state relay for a timed interval (5-20ms). Because the charging current (1A) is negligible compared to the discharge currents (10 - 150A) the capacitor discharges is exponential (approximate square pulse; a 30% drop in voltage is observed when 96 wells are loaded for a 7 ms pulse). Once this cycle is complete the capacitor can be recharged for another pulse or discharged so that the electrodes can be handled safely. All components were purchased from Allied Electronics (Texas,USA). Design plans are available upon request.

Construction of the SDE-device and its cleaning. The SDE-device was designed using the G-Zero Mill software and machined with a Vertical Milling Center (Rapit Output Co., CA, USA). Once machined, the two copper pieces (**Supplementary Figure 2a**) were coated with 5µm of gold by electro deposition and then assembled on a Delrin frame (**Supplementary Figure 2b**) with 12 screws and 4 pins. The Delrin frame matches the dimension of a costar 96-wells plate. The SDE-device was machined with a precision of ± 0.02 mm. The software design files for machining the SDE-device are available upon request.

The SDE-device was cleaned using water and 70% ethanol solution at the end of a series of 96-well electroporations. The device was then sonicated for 5 minutes (Brandson 5200), rinsed with water, dried and sterilized using UV light.

Supplementary References

Heit B. and Kubes P., Sci STKE, 2003.

Tengholm A., Teruel M. N., Meyer T., Sci STKE, 2003.

Ziv N. E., Smith S. J., Neuron, 1996.