Supporting Information for

Photothermal Nanoblade for Large Cargo Delivery into Mammalian Cells

Authors

Ting-Hsiang Wu, Tara Teslaa, Sheraz Kalim, Christopher T. French, Shahriar Maghadam, Randolph Wall, Jeffery F. Miller, Owen N. Witte, Michael A. Teitell* and Pei-Yu Chiou*

*Correspondence: Michael A. Teitell; mteitell@ucla.edu; 675 Charles E. Young Dr. South, MRL 4-762, Los Angeles, CA 90095; TEL: (310) 206-6754; FAX: (310) 267-0382. Pei-Yu Chiou; pychiou@seas.ucla.edu; 420 Westwood Plaza, 37-138, Los Angeles, CA 90095; TEL: (310) 825-8620; FAX: (310) 206-4830.

Figure S-1. Experiment setup and the time-resolved imaging system to quantify bubble. The Ti-coated micropipette was mounted on micromanipulator on an inverted microscope stage. The laser pulse system was a Q-switched, frequency-doubled Nd:YAG laser operated at 532 nm wavelength and 6 ns pulsewidth. The laser beam was split by a polarizing beam splitter with one arm sent into the fluorescence port of the inverted microscope and then through the objective lens (40 $\times$, 0.6 NA), to generate a 260- μ m-wide laser spot on the sample plane. An electrical switch was built to synchronize the excitation laser pulse with the liquid injection system. A time-resolved imaging system was constructed using an intensified CCD camera with exposure times as short as 500 ps. A programmable delay between receiving the laser triggering signal and the camera shutter opening was set by the camera control unit. After the polarizing beam splitter, the other arm of the laser beam was sent through a fluorescent dye cell. The excited fluorescence pulse (wavelength centered $\sim$ 698 nm) was coupled into a multimode fiber and then sent through the microscope condenser to illuminate the sample in synchronization with the camera shutter. A nanosecond time delay between the captured bubble image and the sample excitation laser pulse was achieved by controlling the length of the optical fiber delay line.

Figure S-2. Determining the optimal laser fluence for cargo delivery using photothermal nanoblade. Optimal laser fluence obtained for photothermal cargo transfer using a 1.9 μm wide micropipette tip was 180 mJ/cm^2 . Operating at this threshold laser energy level ensures membrane poration or opening (efficiency = $48 \pm 11\%$ (mean $\pm$ s.d.)) while preserving cell viability ($98 \pm 11\%$). Efficiency was determined by uptake of the membrane-impermeable dye, propidium iodide, present in the media.

Figure S-3. Cell viability and growth post photothermal nanoblade manipulation. (a) Fluorescein-dextran delivery into HeLa cells using the photothermal nanoblade. (Left) Phase contrast image. (Right) Fluorescence image showing fluorescein-dextran (green), propidium iodide (red), and annexin V (blue)

staining 90 min post laser pulsing. Fluorescent imaging shows that 19 out of a total number of 40 cells were successfully loaded with fluorescein-dextran (consistent with 50% membrane opening efficiency at a laser fluence of 180 mJ/cm²) and all the cells remained viable, as shown by the exclusion of annexin V and propidium iodide stains. (b) Monitoring laser pulsed cells over 24 hours shows that cells stay viable and continue to grow and divide. Cells were cultured on a patterned substrate to confine cell adhesion and growth within a pre-defined circular region. Every cell within the circular region was subjected to laser pulsing and membrane opening by pulsing the Ti-coated micropipette at 0 hour.

Figure S-4. Robust repeated operation of the photothermal nanoblade. An explosive vapor bubble (imaged at 70 ns after laser pulse illumination) at the micropipette tip can be repeatedly produced >50 times without showing significant bubble size alteration or Ti coating degradation. The micropipette was mounted on a micromanipulator with the tip suspended in water.

Experiment	Bacteria concentration per ml	No. of red fluorescent cells	No. of cells with detectable intracellular bacteria	Injection efficiency (%)
No. 1	4×10^8	17	4	23.5
No. 2	6.5×10^8	6	5	83.3
No. 3	6.5×10^8	10	3	30

Table S-1. Bacteria injection efficiency evaluation. *B. thailandensis* were co-injected with red fluorescent dextran-tetramethylrhodamine (molecular weight 40k Da., 200 mg/ml in 1× PBS, pH 7.4) into HeLa cells using a Ti-coated photothermal micropipette. Injection efficiency was calculated as the

number of cells with intracellular bacteria divided by the number of cells injected with the red fluorescent dye.

Movie S-1. Photothermal nanoblade delivery into HEK293T cells. Real-time photothermal nanoblade delivery into 3 cells consecutively. Injected liquid expansion of each cell can be seen immediately after each laser pulse (white flash). Positioning the micropipette tip on the cell membrane and laser pulse irradiation with 1-5pL liquid injection takes ~3 sec per cell.

Movie S-2. 3D FDTD numerical simulation of the electromagnetic intensity pattern on a Ti-coated micropipette. The plane wave excitation on a Ti-coated glass micropipette tip was simulated. Panels show the intensity ($|E^2|$) on the tip of the micropipette under plane wave excitation with linear and circular polarizations. The elapsed time between frames was 1/28 of one wave oscillation period.