Supporting Information for

Photothermal Intracellular Delivery Using Gold Nanodisk Arrays

Chuanzhen Zhao,[1,2] Tianxing Man,[3] Xiaobin Xu,[1,2,4] Qing Yang,[1,2] Wenfei Liu,[1,2]

Steven J. Jonas,[2,5] Michael A. Teitell,[2,6,7] Pei-Yu Chiou,[*,3,6] and Paul S. Weiss[*,1,2,6,8]

[1]Department of Chemistry and Biochemistry, University of California, Los Angeles, Los Angeles, California 90095, United States

[2]California NanoSystems Institute, University of California, Los Angeles, Los Angeles, California 90095, United States

[3]Department of Mechanical and Aerospace Engineering, University of California, Los Angeles, Los Angeles, California 90095, United States

[4]School of Materials Science and Engineering, Tongji University, Shanghai 201804, China

[5]Department of Pediatrics, David Geffen School of Medicine, Eli & Edythe Broad Center of Regenerative Medicine and Stem Cell Research, Children's Discovery and Innovation Institute, University of California, Los Angeles, Los Angeles, California 90095, United States

[6]Department of Bioengineering, University of California, Los Angeles, Los Angeles, California 90095, United States

[7]Department of Pathology and Laboratory Medicine, Jonsson Comprehensive Cancer Center, Eli & Edythe Broad Center of Regenerative Medicine and Stem Cell Research, and Molecular Biology Institute, University of California, Los Angeles, California 90095, United States

[8]Department of Materials Science and Engineering, University of California, Los Angeles, Los Angeles, California 90095, United States

*Email: pychiou@gmail.com and psw@cnsi.ucla.edu

Materials and Methods

Materials. Prime quality 4″ silicon (Si) wafers (P/B, 0.001-0.005 Ω·cm, thickness 500 μm) were purchased from Silicon Valley Microelectronics, Inc. (Santa Clara, CA, USA). Sylgard 184® silicone elastomer kits were purchased from Ellsworth Adhesives (Germantown, WI, USA). Iron nitrate, and thiourea were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as received. Deionized water (18.2 MΩ·cm) from a Milli-Q system (Millipore, Billerica, MA) was used in all experiments.

Chemical Lift-Off Lithography. Gold nanodisk substrates were prepared *via* chemical lift-off lithography (CLL).[S1,S2] Briefly, a CHA solution electron-beam evaporator was used to first deposit a 10-nm-thick titanium (Ti) adhesion layer followed by a 30-nm gold (Au) film onto desired substrates. The substrates were then annealed in a hydrogen flame for ~10 s before immersion into 1 mM 11-mercapto-1-undecanol solution for over 12 h. Polydimethylsiloxane (PDMS) stamps[S1] were exposed to oxygen plasma (Harrick Plasma, Ithaca, NY) for 40 s at a power of 18 W and a pressure of 10 psi for activation before contact with the substrate for over 12 h. After the PDMS stamps were lifted-off, the substrate was then immersed into an aqueous solution of 20 mM iron nitrate and 30 mM thiourea for 30 min to etch the Au film selectively within the exposed area. The etch rate was ~1 nm/min. Substrates were then rinsed with deionized water, dried under a stream of N_2 before use, and sterilized *via* ultraviolet (UV) irradiation overnight.

Characterization. Scanning electron microscope (SEM) images were obtained using a Zeiss Supra 40VP scanning electron microscope with an Inlens SE Detector (Inlens secondary electron detector). Atomic force microscope (AFM) imaging was performed on a Bruker FastScan system using peak force tapping mode with ScanAsyst-Air tips. Optical images were taken with a Zeiss Axiotech optical microscope. Fluorescence images were taken with an upright fluorescence microscope (Axio Scope.A1, Carl Zeiss) equipped with a 10X objective lens.

Cell Seeding and Culture on Substrate. HeLa cells (ATCC) were maintained in Dulbecco's modified essential medium (DMEM, Corning) supplemented with 10% (vol/vol) fetal bovine

serum (FBS, Thermo Scientific), 1% penicillin/streptomycin (Mediatech), and 1% sodium pyruvate (Corning). Chips were coated with fibronectin (Sigma, 40 μg/mL in phosphate buffered saline) to promote cell adhesion. HeLa cells were seeded on to the chip and kept in an incubator at 37 °C and 5% CO_2 for 24 h. Cells were stained with 1 μg/mL Calcein AM (Invitrogen) to show cell seeding results.

Cell Fixation. Chips with HeLa cells were rinsed with phosphate buffered saline (PBS) three times and soaked in 4% paraformaldehyde in PBS for 20 min at room temperature. The chips were then removed from the paraformaldehyde solution and were then rinsed with PBS three times prior to immersion in ethanol of graded concentrations (50%, 70%, 90%, and 100%) for 5 min each. And then hexamethyldisilazane (HMDS) three times for 7 min each. Samples were then air dried overnight prior to analysis.

Laser-Scanning and Delivery Setup. A Q-switched Nd:YAG laser (Minilite I, Continuum) with wavelength of 532 nm, beam diameter of 3 mm, and pulse duration of 6 ns was used to scan each sample. A half-wave polarizer and polarizing beam splitter designed for a 532 nm laser was used to adjust the power splitting ratio of the two beams. Laser energy was checked prior to each experiment using a laser energy meter (Nova II, Ophir). Sample were placed on an automated X-Y translation stage to expose their entire 1 cm^2 area to the laser beam (Figure S1). As shown in the schematic, the laser beam was directed from the top of the sample. We used 35-mm petri dishes to hold the sample in the center during the laser pulsing, which mitigates the effect of the meniscus.

The laser beam is 3 mm in diameter, while the HeLa cell sizes are in the range of tens of microns. In our experiments, cells were treated with single laser pulses to obtain the current results.

Fluorescence Microscopy and Cell Counting. Cells were incubated in 37 °C and 5% CO_2 for 90 min before checking delivery efficiency and viability. The cell permeable nuclear dye Hoechst 33342 was used to label all processed cells for ease of quantifying the total number of cells present on each chip. HeLa cells that retained the cell-impermeable calcein molecules represented successful delivery. Cell viability was determined using propidium iodide (5 µg/mL), which is permeable to dead cells. Cell number was determined using both the automatic counting algorithm of the Fiji image processing software package and manual counting to double check. Each data point in **Figure 5e-g** represents the mean value of at least 3 randomly selected fields of view with at least 200 cells; error bars represent standard deviation.

Numerical Simulations. Temperature distributions from the plasmonic structures were modeled using a finite element method (COMSOL, Multiphysics 5.3) to calculate substrate-light interactions. The geometry was constructed based on the dimensions of the actual nanodisk chips. Electromagnetic wave with linear polarization in diagonal direction was applied, which is consistent with experimental configurations. Electromagnetic interaction was calculated using a scattered field formulation method in the z direction (wavelength = 532 nm, fluence = 11 mJ/cm^2) and the resistive loss was used as the heat source for transient heat transfer. Perfectly matched layers were applied to truncate the modeling domain.

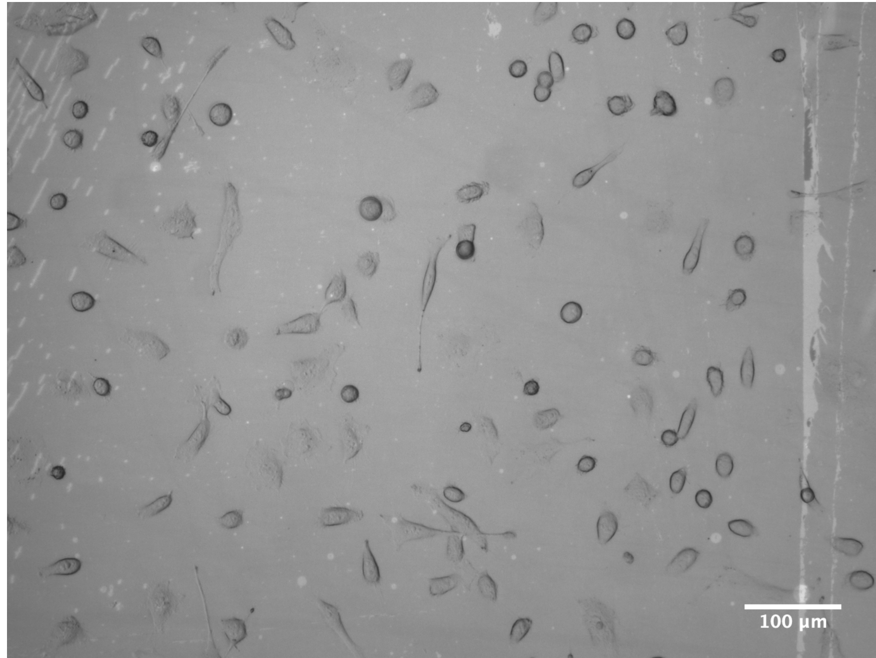

Figure S1. Optical microscope image of cells on 350-nm Au nanodisk arrays. The Au nanodisks are not visible in the optical microscope due to their sub-micron features.

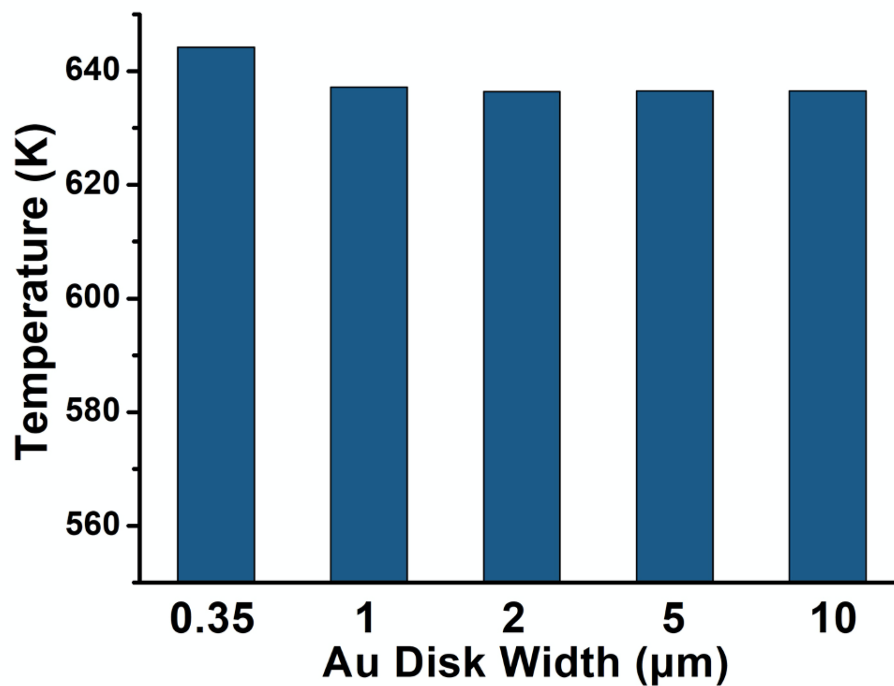

Figure S2. Simulation results tip temperature on different dimensions of Au nanodisk arrays upon laser radiation of 11 mJ/cm^2.

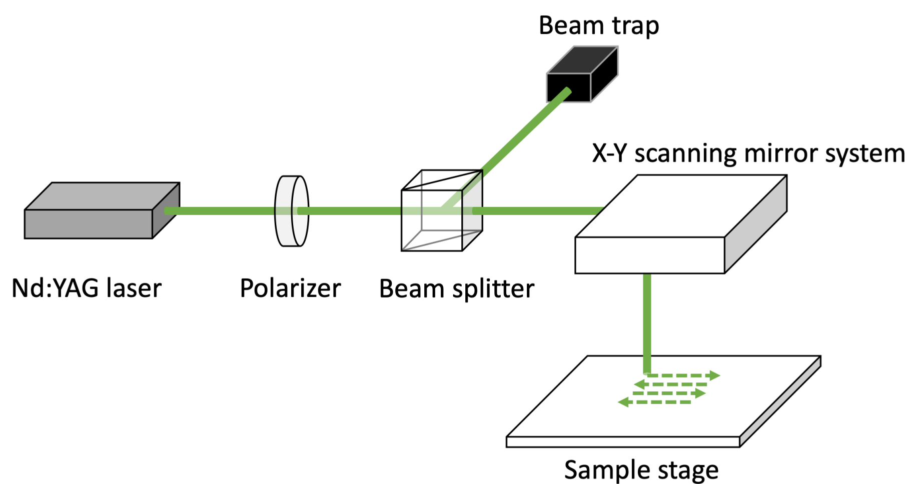

Figure S3. Schematic diagram of the optical setup.

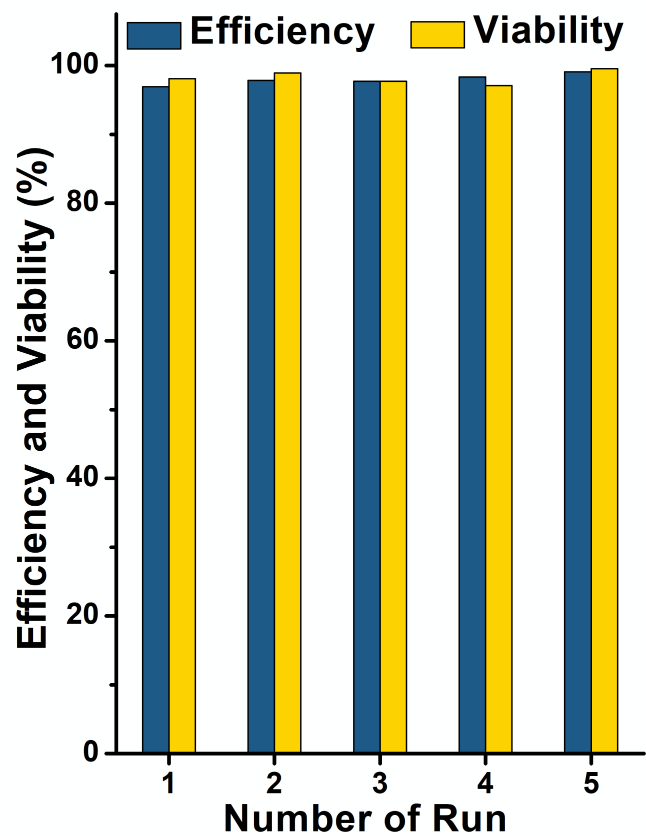

Figure S4. Delivery efficiency and cell viability results of 0.6 kDa calcein AM using a 1-μm-wide gold (Au) nanodisk arrays chip after five runs under 11 mJ/cm^2 laser fluence.

References

(S1) Zhao, C.; Xu, X.; Bae, S. H.; Yang, Q.; Liu, W.; Belling, J. N.; Cheung, K. M.; Rim, Y. S.; Yang, Y.; Andrews, A. M.; Weiss, P. S. Large-Area, Ultrathin Metal-Oxide Semiconductor Nanoribbon Arrays Fabricated by Chemical Lift-Off Lithography. *Nano Lett.* **2018**, *18*, 5590–5595.

(S2) Zhao, C.; Xu, X.; Chiang, N.; Yang, Q.; Liu, W.; Schwartz, J. J.; Andrews, A. M.; Weiss, P. S. Two-Dimensional Plasmonic Nanostructure Arrays Fabricated by Double-Patterning Chemical Lift-Off Lithography. In preparation.