Plasmon-enhanced ultrafast laser cell transfection

Eric Diebold

Harvard University

Outline

Cell Transfection

Plasmon-enhanced laser cell transfection

Measurement of transfection efficiency using fluorescence-activated cell sorting (FACS)

Background: femtosecond laser cell transfection

Cell transfection: "infection by transformation"

Introduction of biological material into a cell, resulting in a modification of its genetic composition

Background: femtosecond laser cell transfection

Cell transfection is central to:

Genetic engineering

Potential gene therapies - DNA, siRNA, etc.

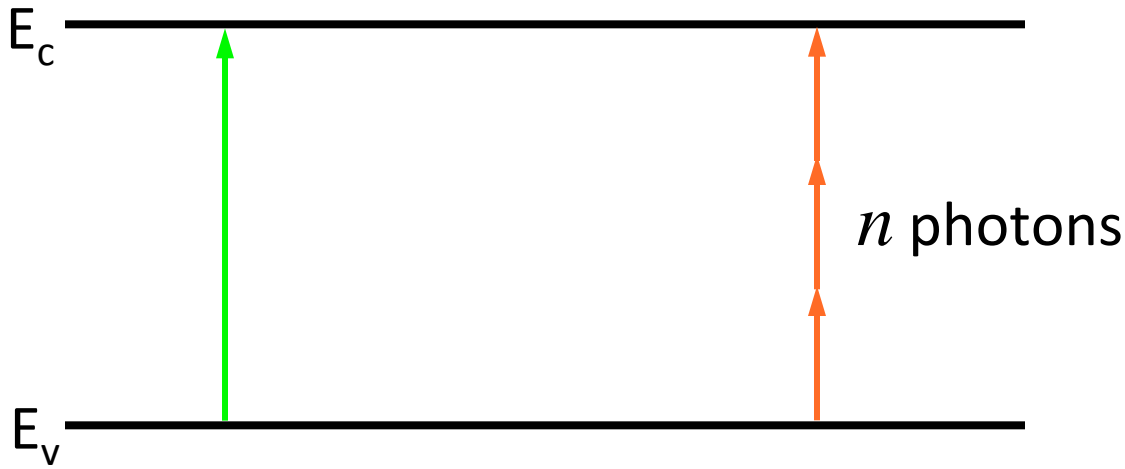
Basic biological research

Background: femtosecond laser cell transfection

Transfection technique	Efficiency	Toxicity	Throughput
Lipid reagents	Medium to High	Medium	High
Electroporation	Low to Medium	High	High
Microinjection	High	Low	Low
Fs-laser transfection	Medium to High	Low	Low

Background: femtosecond laser cell transfection

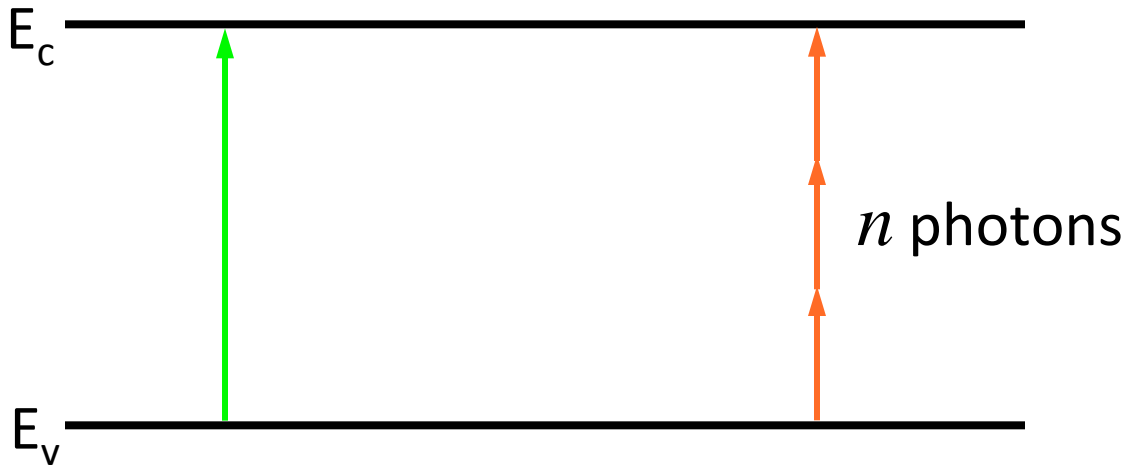
Linear vs. nonlinear absorption



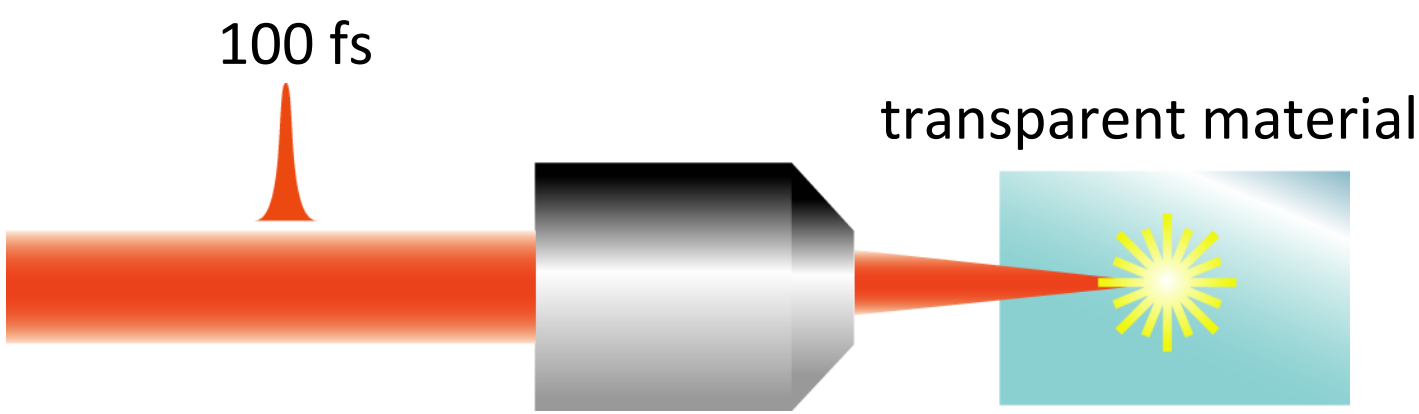
$$N_{abs} \propto \sigma_1 I^1 \qquad N_{abs} \propto \sigma_n I^n$$

Background: femtosecond laser cell transfection

Linear vs. nonlinear absorption

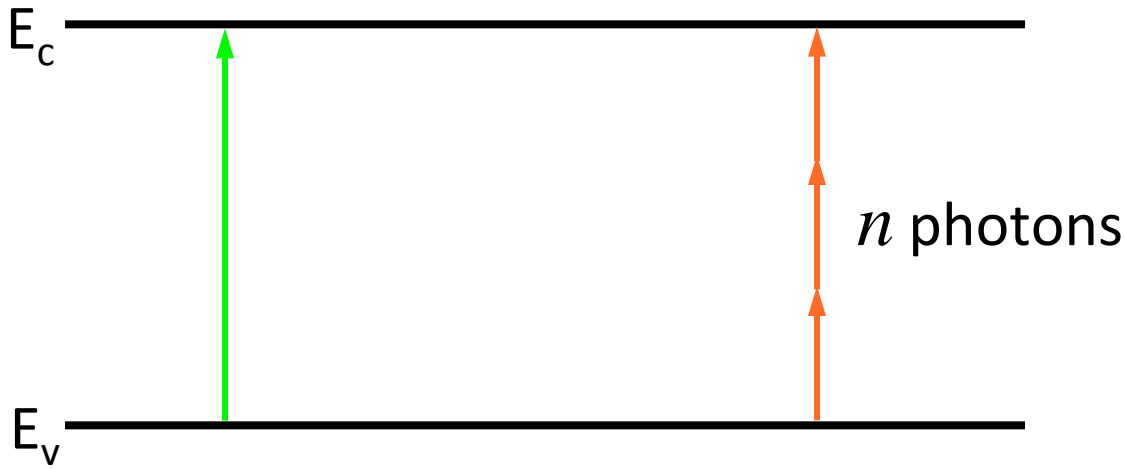


$$N_{abs} \propto \sigma_1 I^1 \qquad N_{abs} \propto \sigma_n I^n$$



Background: femtosecond laser cell transfection

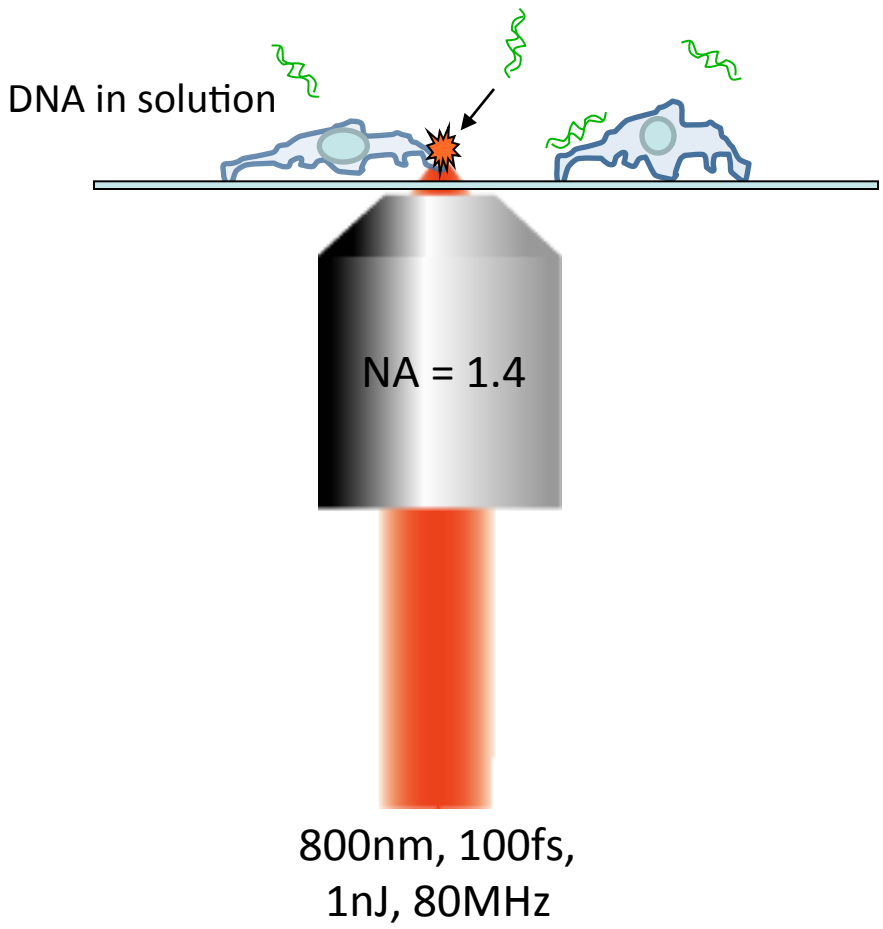
Linear vs. nonlinear absorption

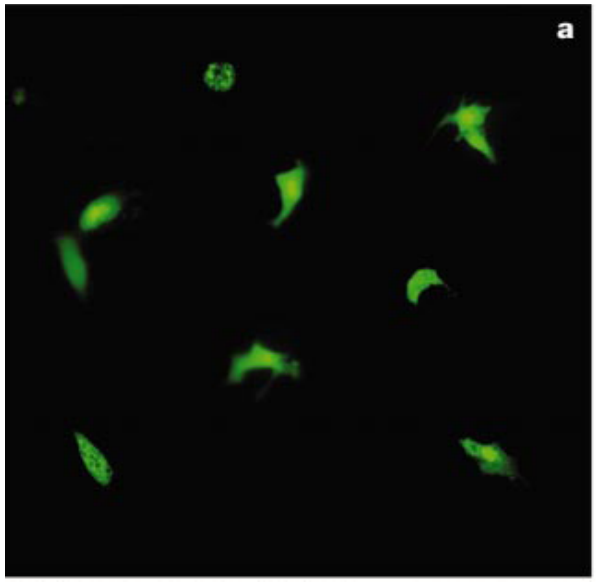


$$N_{abs} \propto \sigma_1 I^1 \qquad N_{abs} \propto \sigma_n I^n$$

transparent material

Background: femtosecond laser cell transfection





Transfection of cells
with near-100% efficiency

Excellent efficiency, but terrible
throughput!

U. Tirlapur and K. Konig, *Nature* **418**, 290-291 (2002)

Outline

Cell Transfection

Plasmon-enhanced laser cell transfection

Measurement of transfection efficiency using fluorescence-activated cell sorting (FACS)

Plasmonic substrates

Use sub-wavelength focusing properties of plasmonic nanostructures to replace high-NA focusing.

Plasmonic substrates

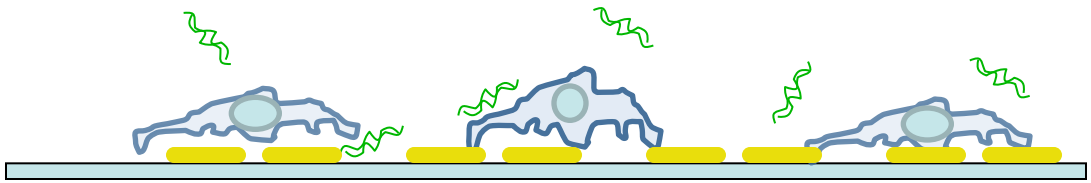
Use sub-wavelength focusing properties of plasmonic nanostructures to replace high-NA focusing.

Larger laser pulse energies combined with larger spot sizes and/or scanning, many cells can be transfected quickly.

Plasmonic substrates

Use sub-wavelength focusing properties of plasmonic nanostructures to replace high-NA focusing.

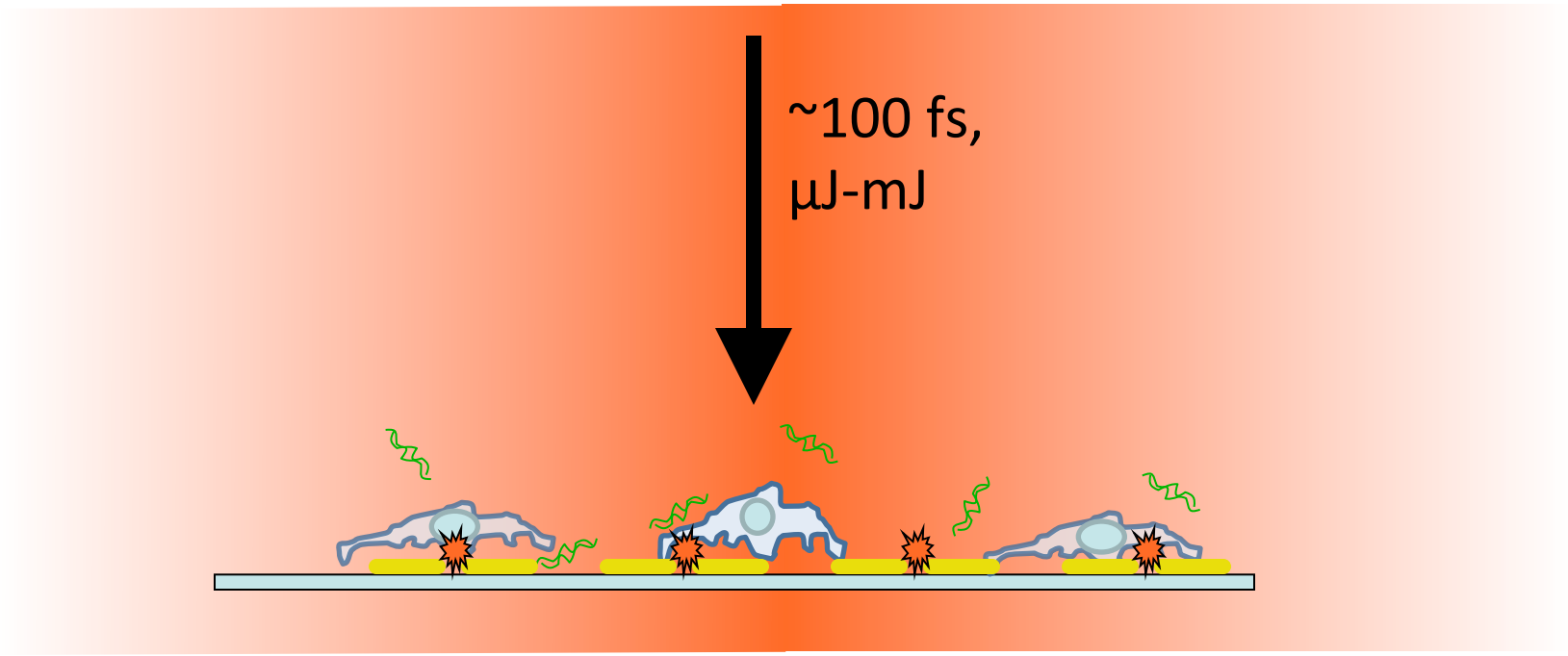
Larger laser pulse energies combined with larger spot sizes and/or scanning, many cells can be transfected quickly.



Plasmonic substrates

Use sub-wavelength focusing properties of plasmonic nanostructures to replace high-NA focusing.

Larger laser pulse energies combined with larger spot sizes and/or scanning, many cells can be transfected quickly.



Plasmonic substrates

The choice of plasmonic substrate has specific requirements:

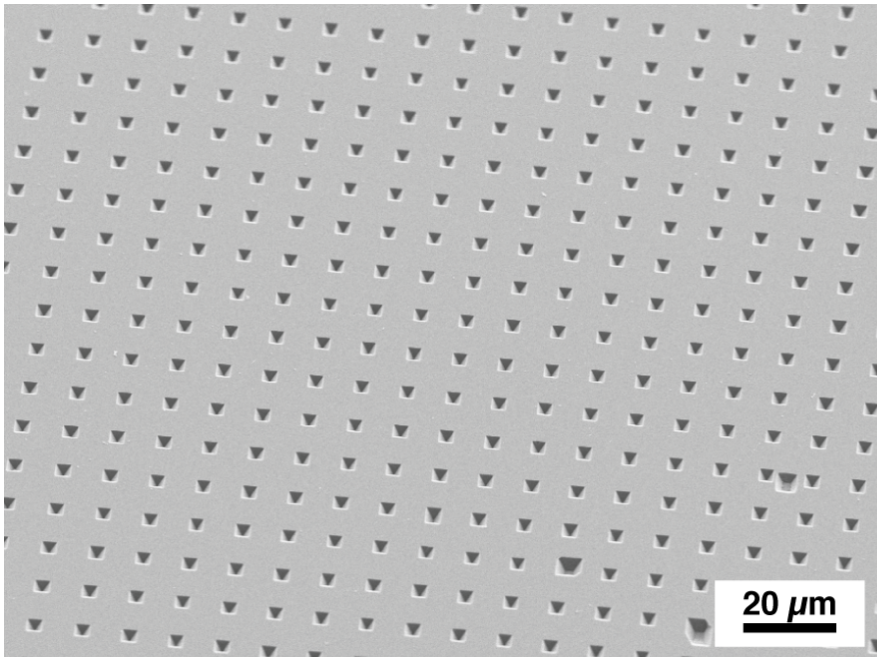
1. Field enhancement must be in close proximity to cell membrane.

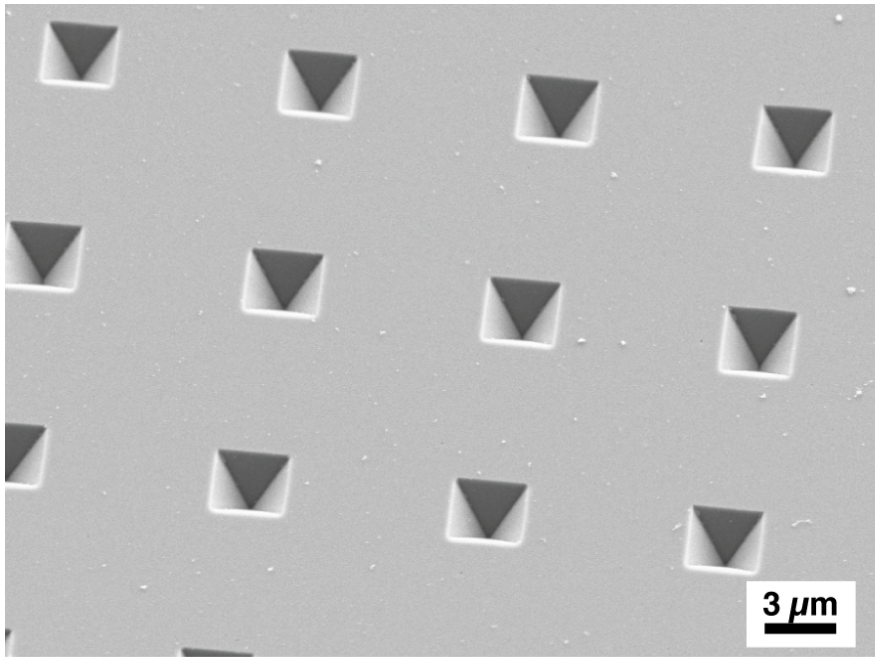
2. Field enhancing regions (areas of damage) must be accessible to DNA, RNA, etc. in surrounding solution.

3. Substrate fabrication method must be scalable in size.

Plasmonic substrates

Photolithography + KOH anisotropic etching

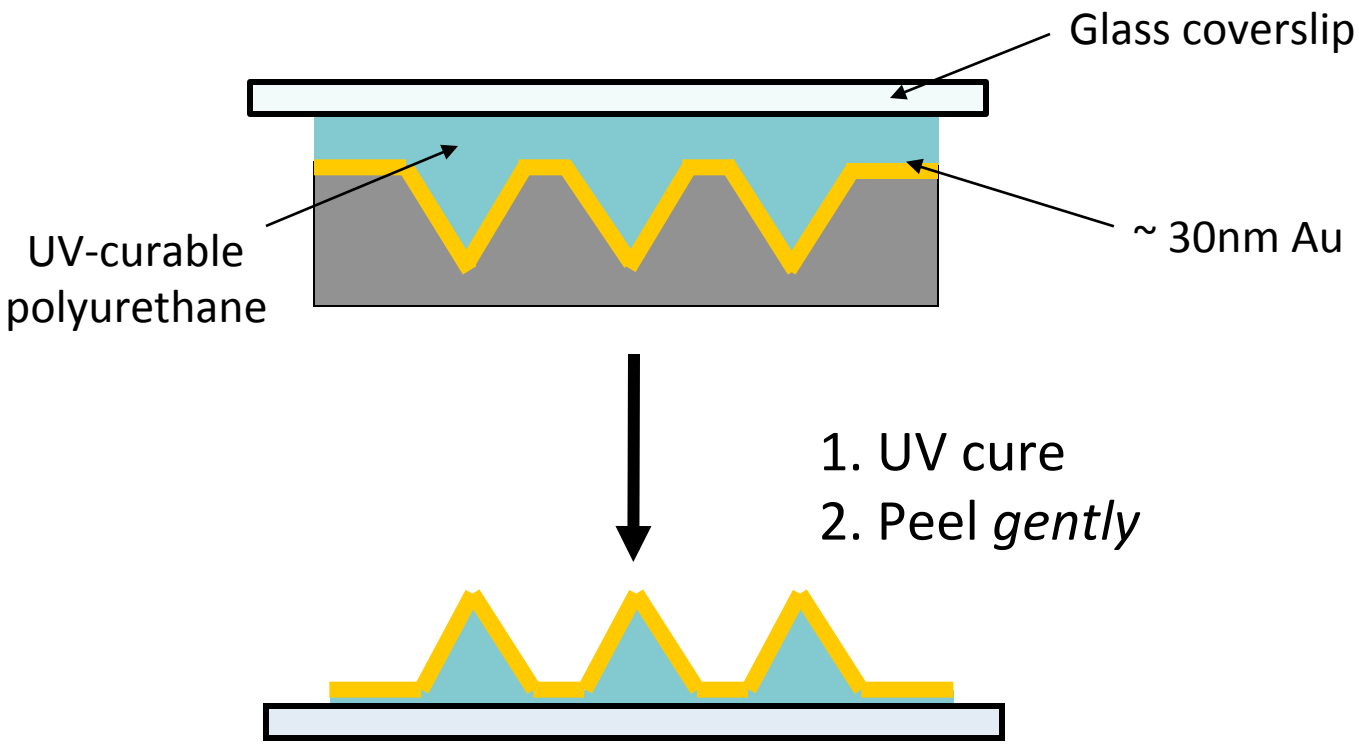




Plasmonic substrates

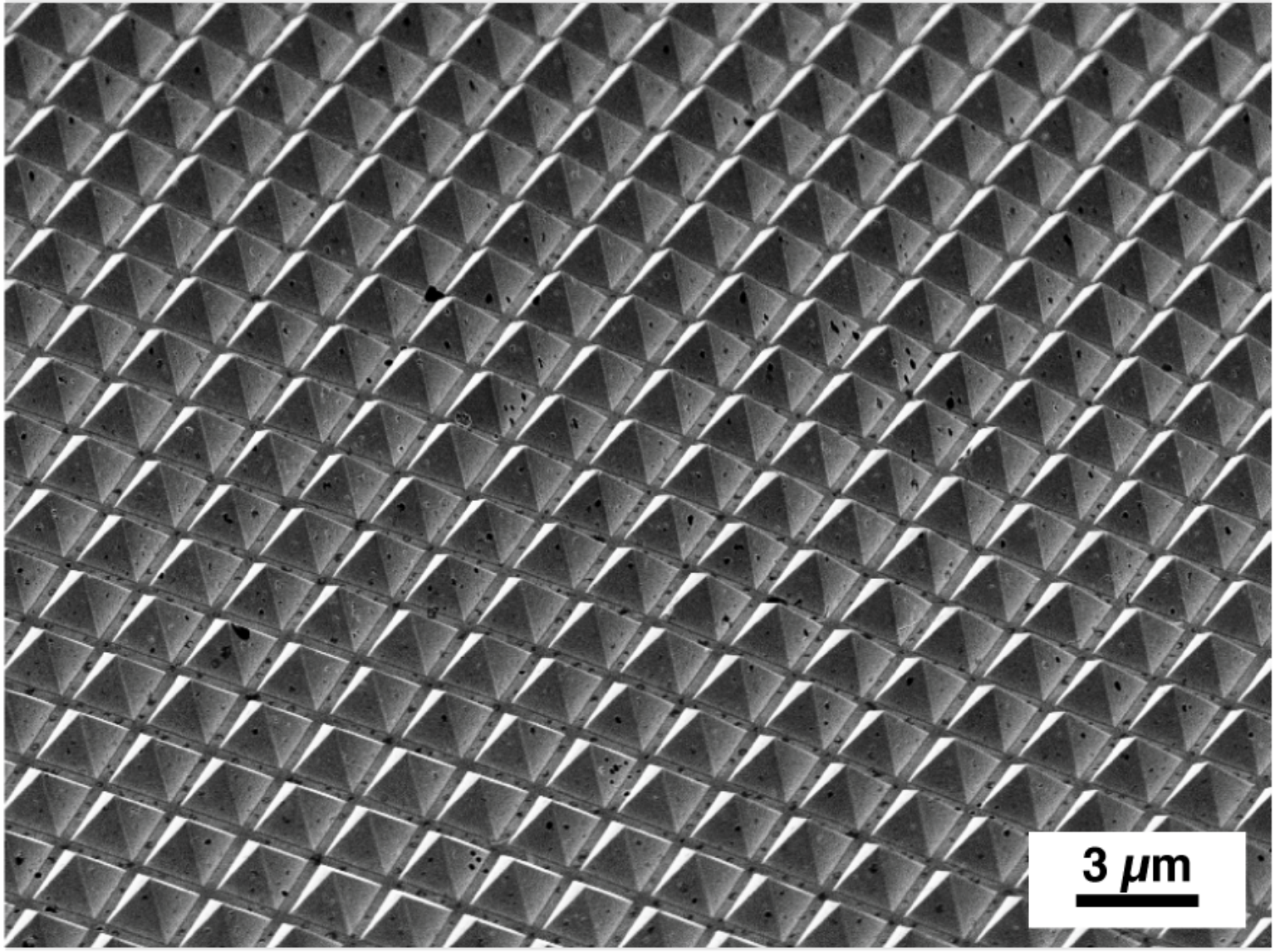
Substrate: template-stripped gold pyramid array

Template stripping exploits poor adhesion of noble metals on SiO_2



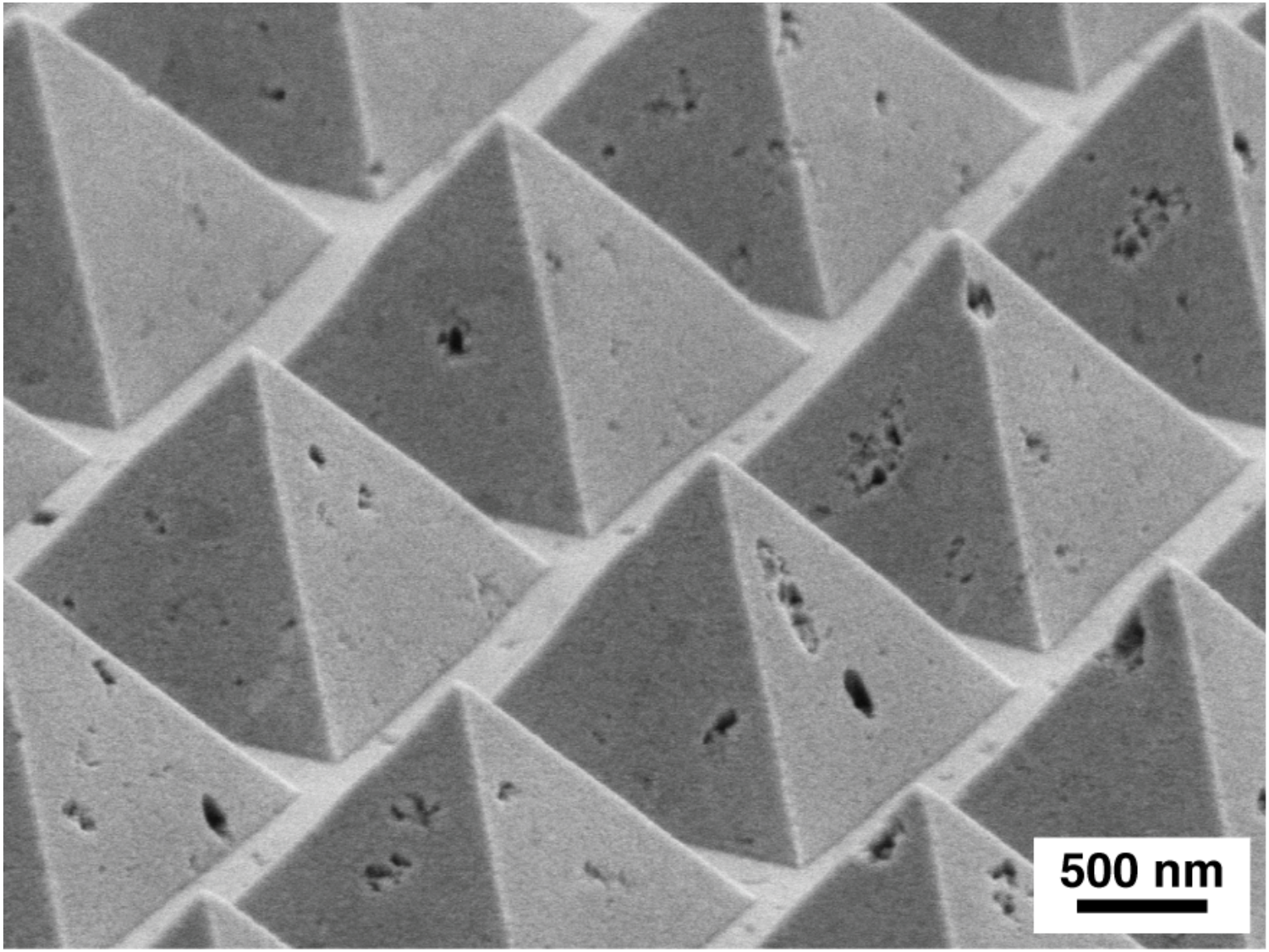
P. Nagpal et al. *Science* **325**, 594 (2009)

Plasmonic substrates



30nm Au on polyurethane

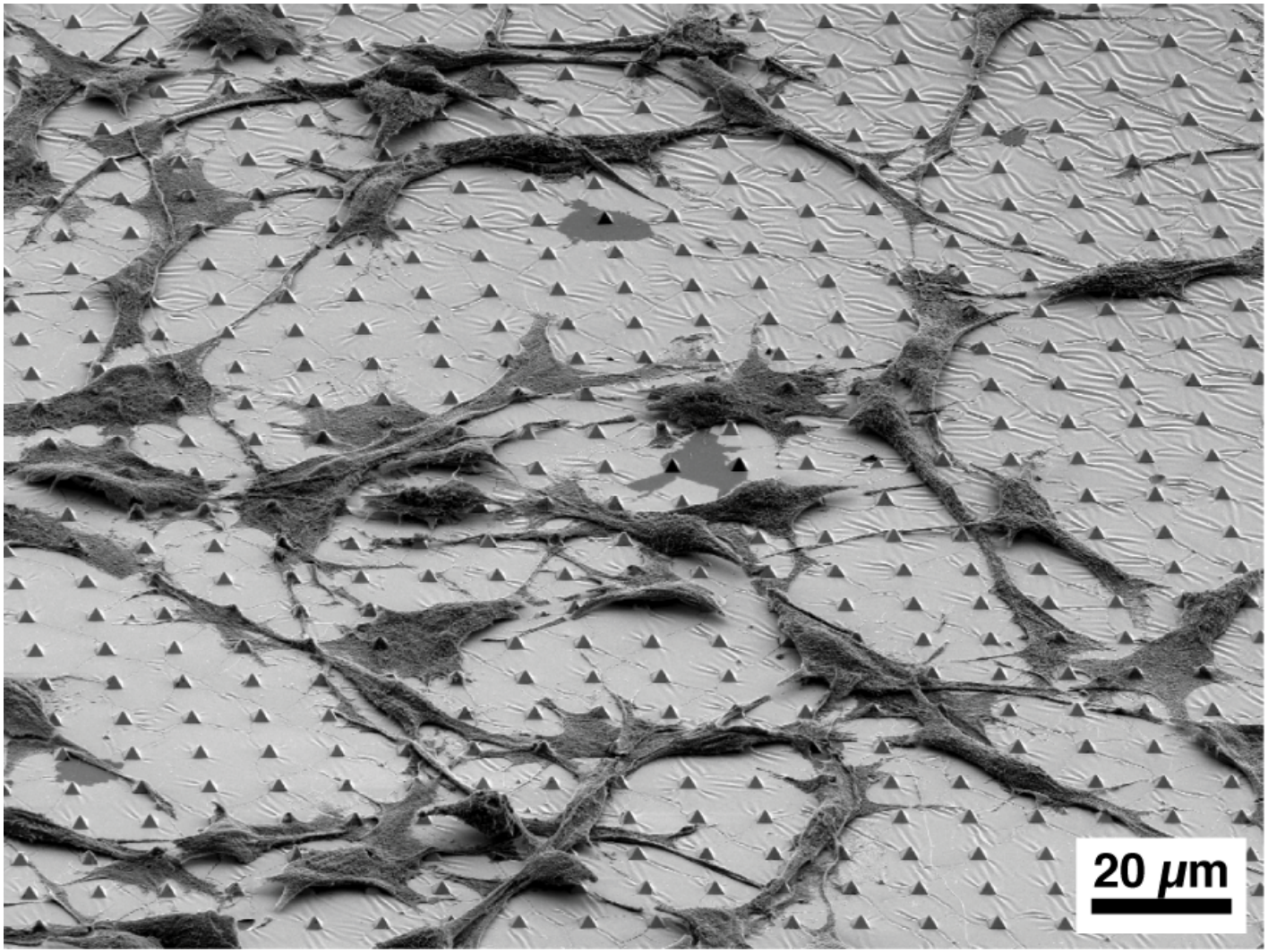
Plasmonic substrates



30nm Au on polyurethane

Plasmonic substrates

Biocompatibility of pyramidal substrates



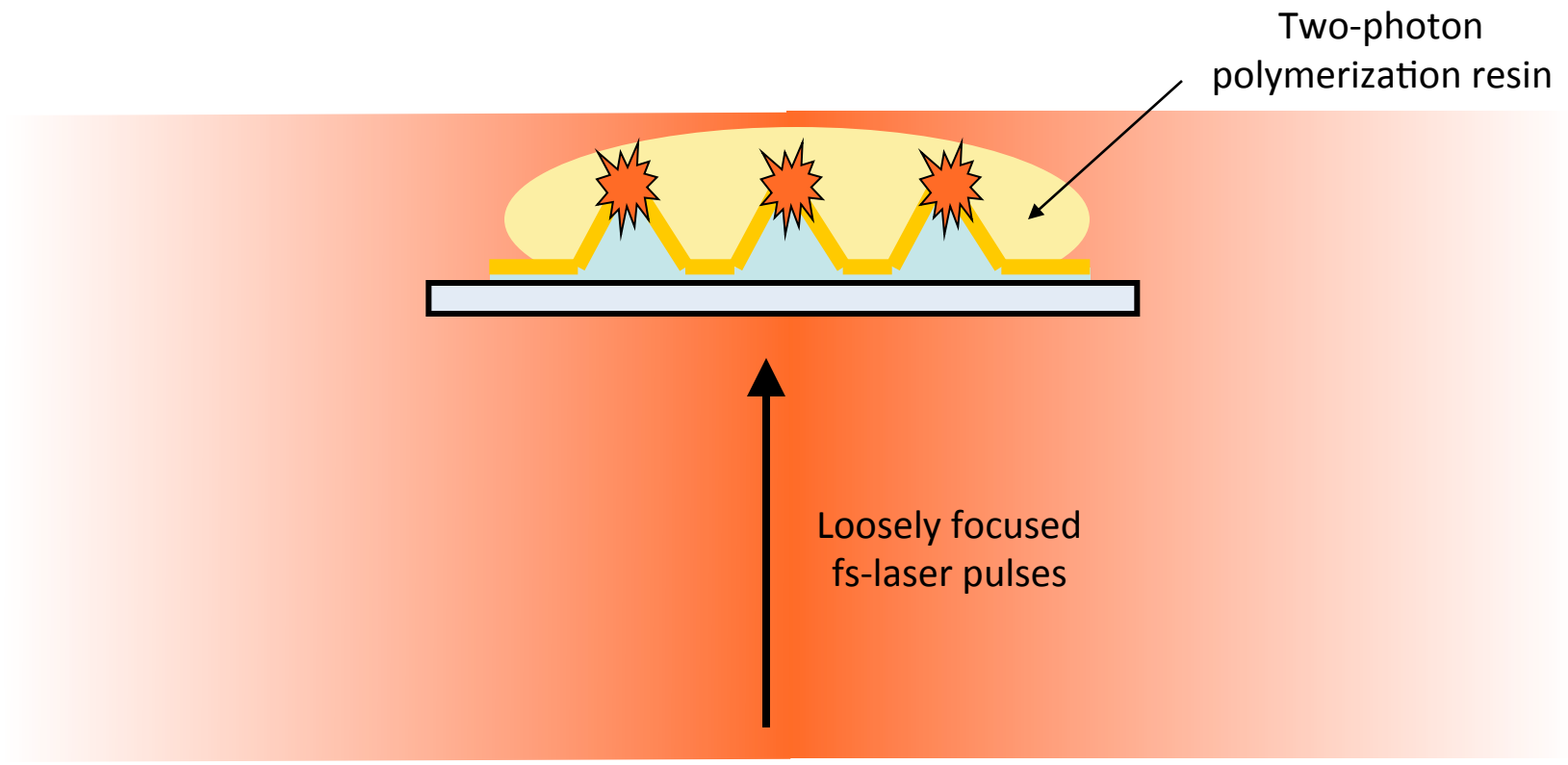
Plasmonic substrates

Where is the field enhancement?

Plasmonic substrates

Where is the field enhancement?

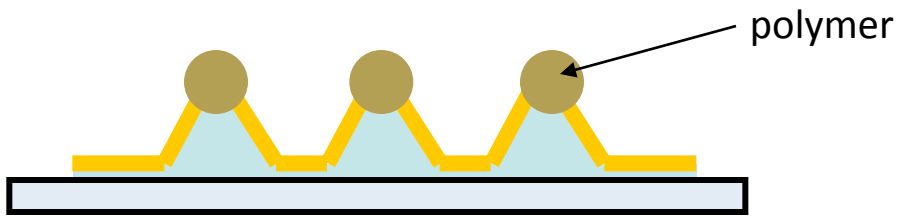
Two-photon absorption polymerization is an intensity-dependent nonlinear effect. Polymerization occurs where the field is most intense.



Plasmonic substrates

Where is the field enhancement?

Two-photon absorption polymerization is an intensity-dependent nonlinear effect. Polymerization occurs where the field is most intense.

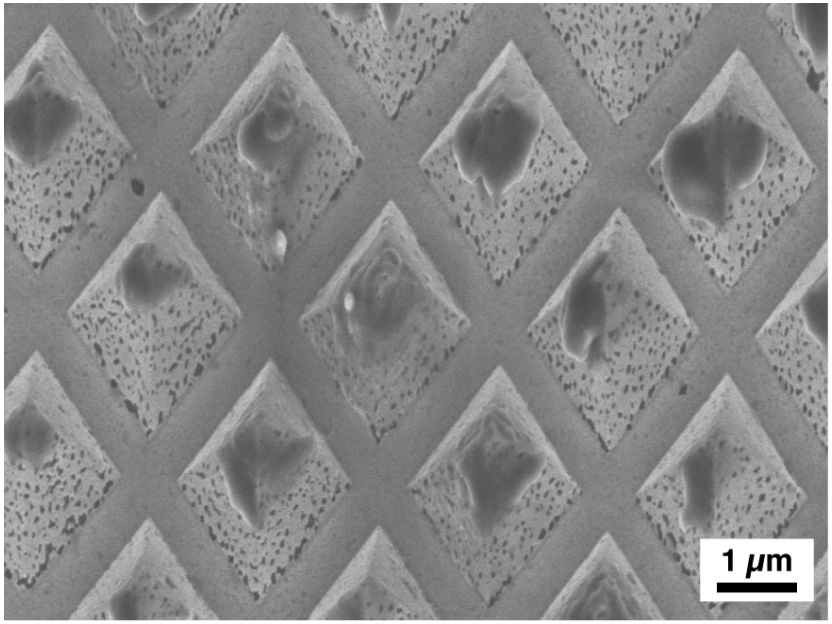


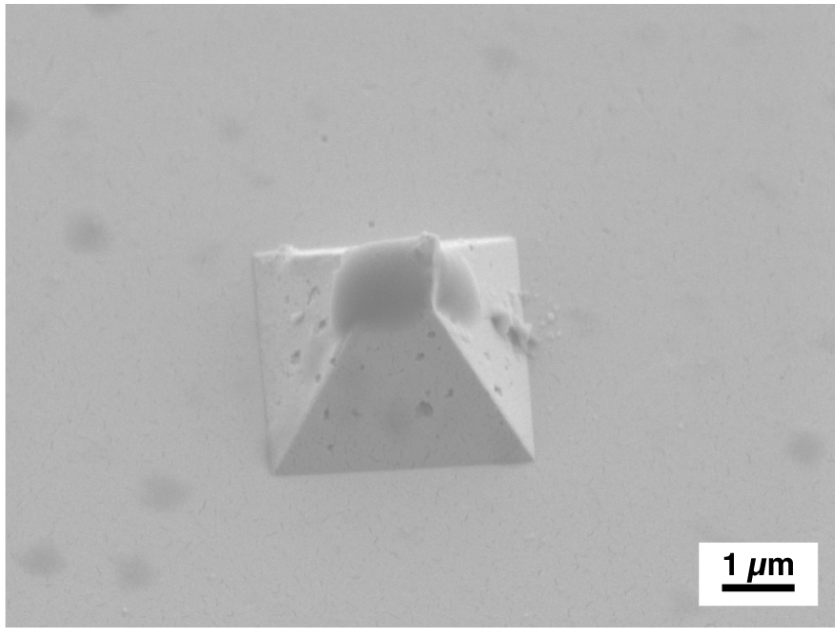
after washing in ethanol

Plasmonic substrates

Where is the field enhancement?

Two-photon absorption polymerization is an intensity-dependent nonlinear effect. Polymerization occurs where the field is most intense.

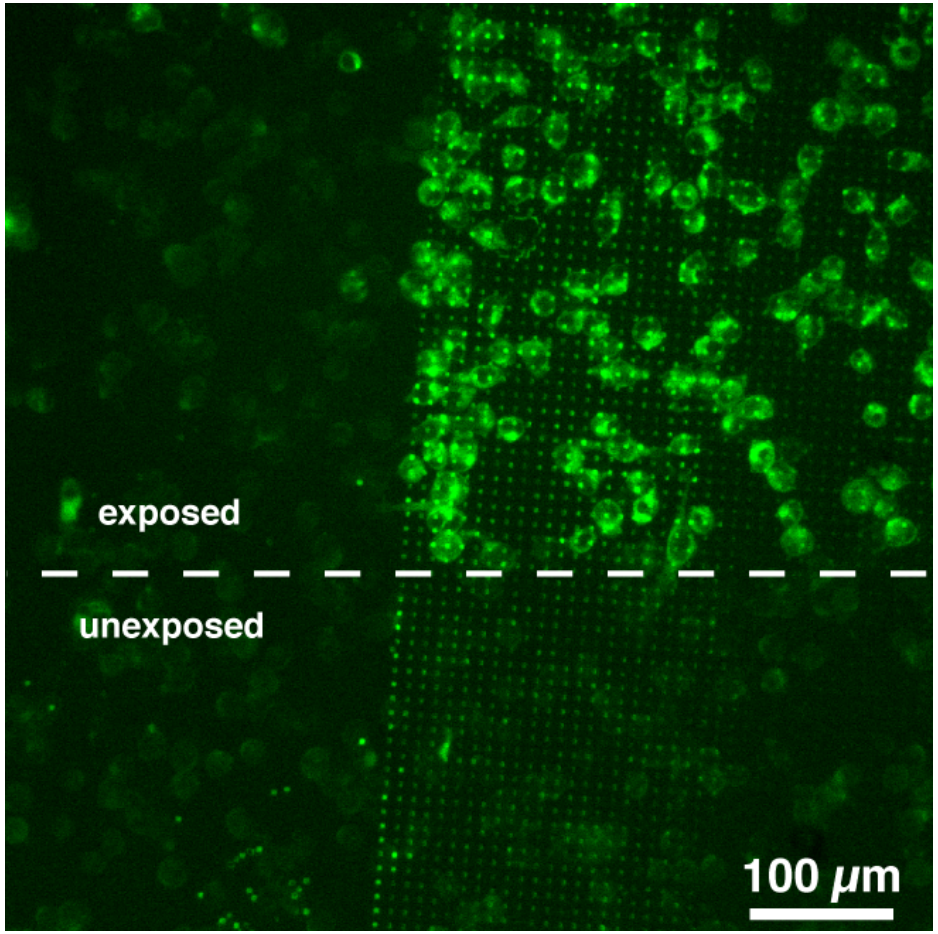




Plasmon-enhanced transfection

Plasmon-enhanced cell perforation

Exposing substrate with femtosecond pulse train allows cellular introduction of lipid-sensitive dye (FM1-43) only on pyramidal region.

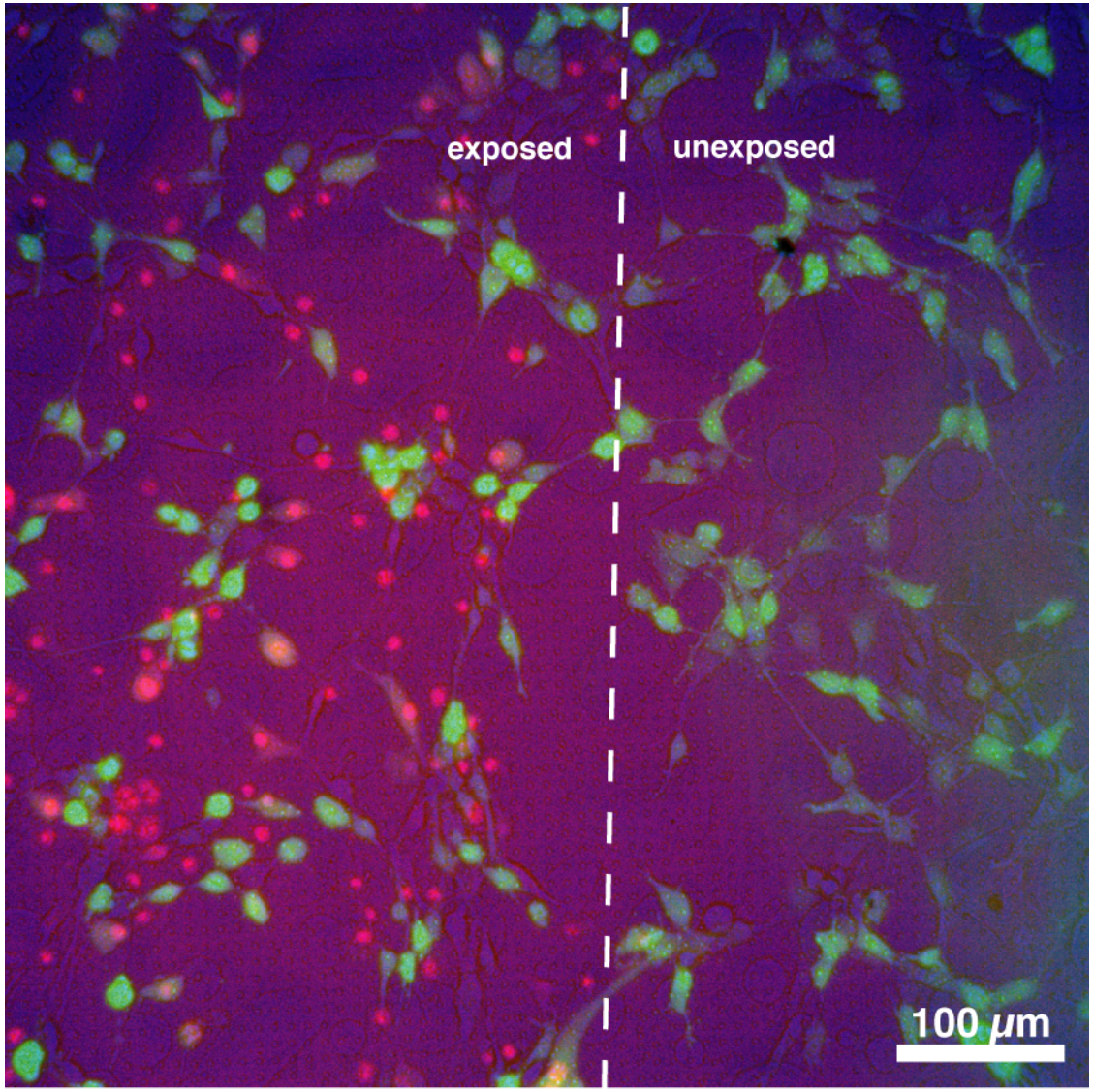


Cell line:
Human thymus

Laser spot size diameter ~ 30 μm

Plasmon-enhanced transfection

Transfection using rhodamine-labeled siRNA



Cell line:
NM2GFP (ATCC)

Image overlay:

Blue – brightfield

Green – GFP fluorescence

Red – Rhodamine fluorescence

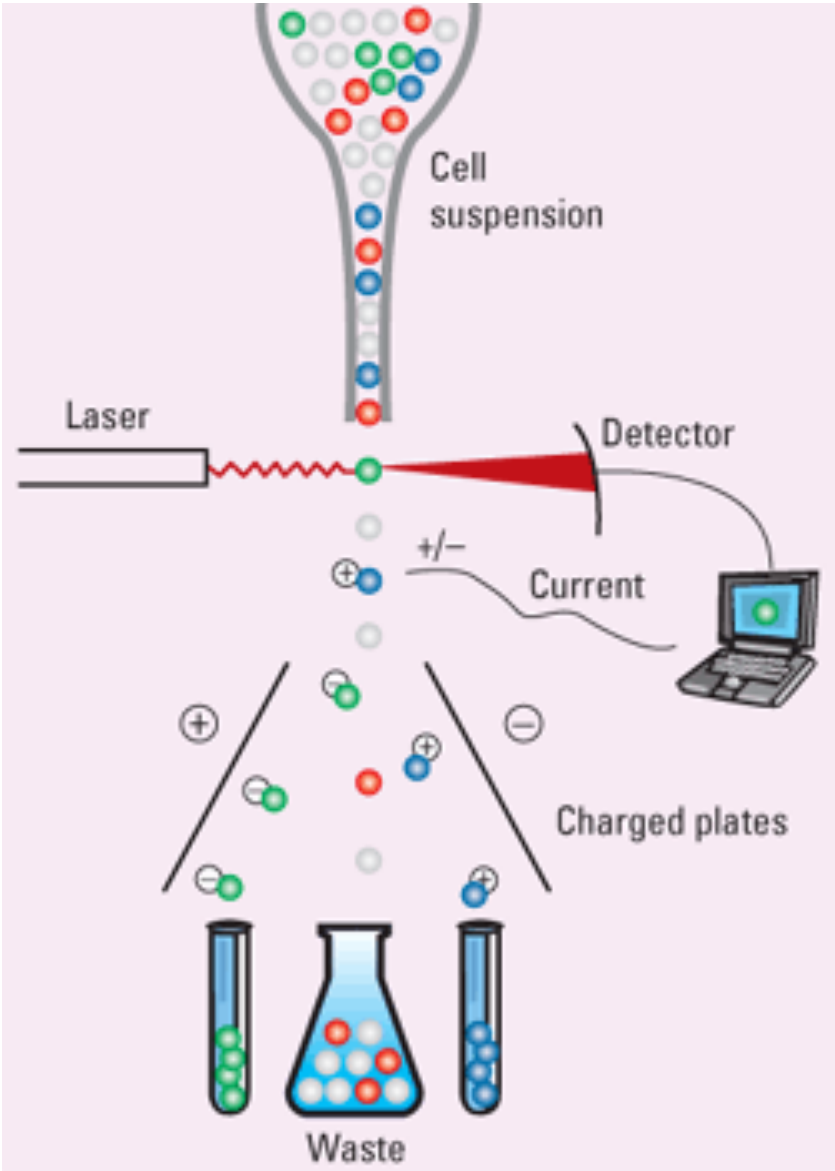
Outline

Cell Transfection

Plasmon-enhanced laser cell transfection

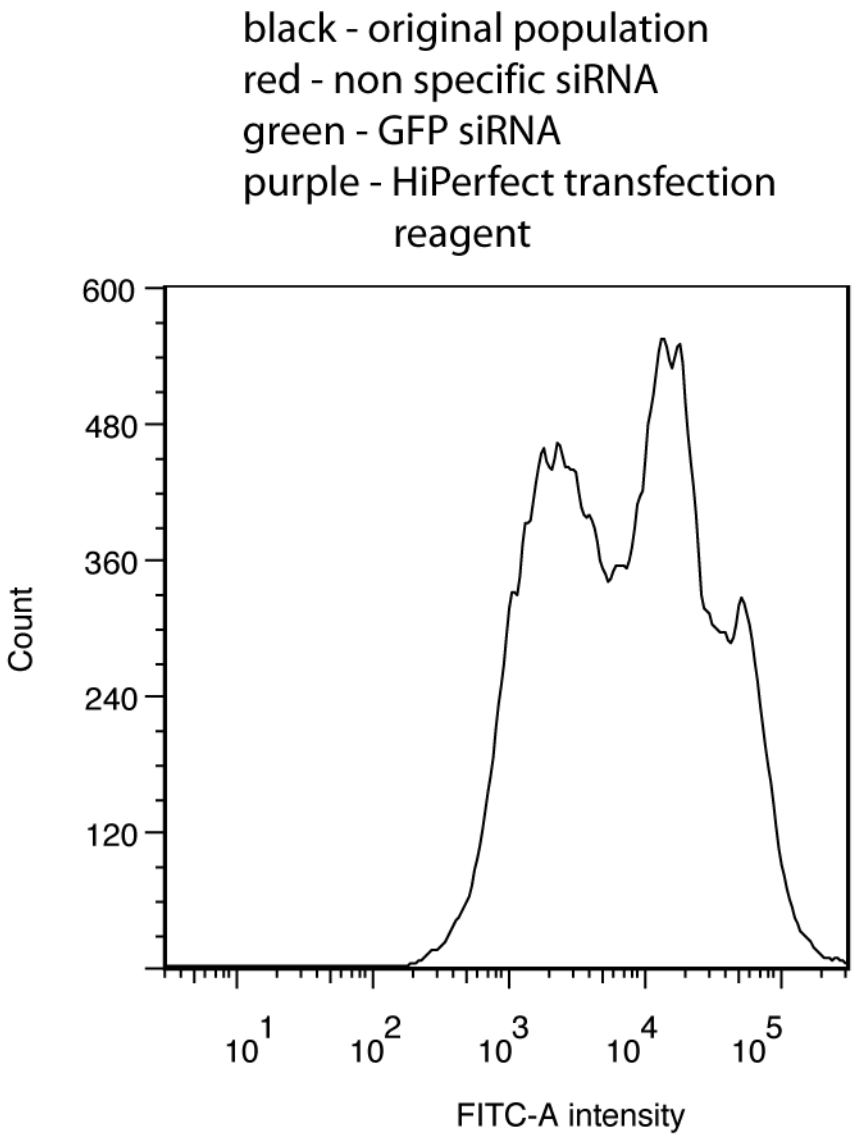
Measurement of transfection efficiency using fluorescence-activated cell sorting (FACS)

FACS transfection analysis



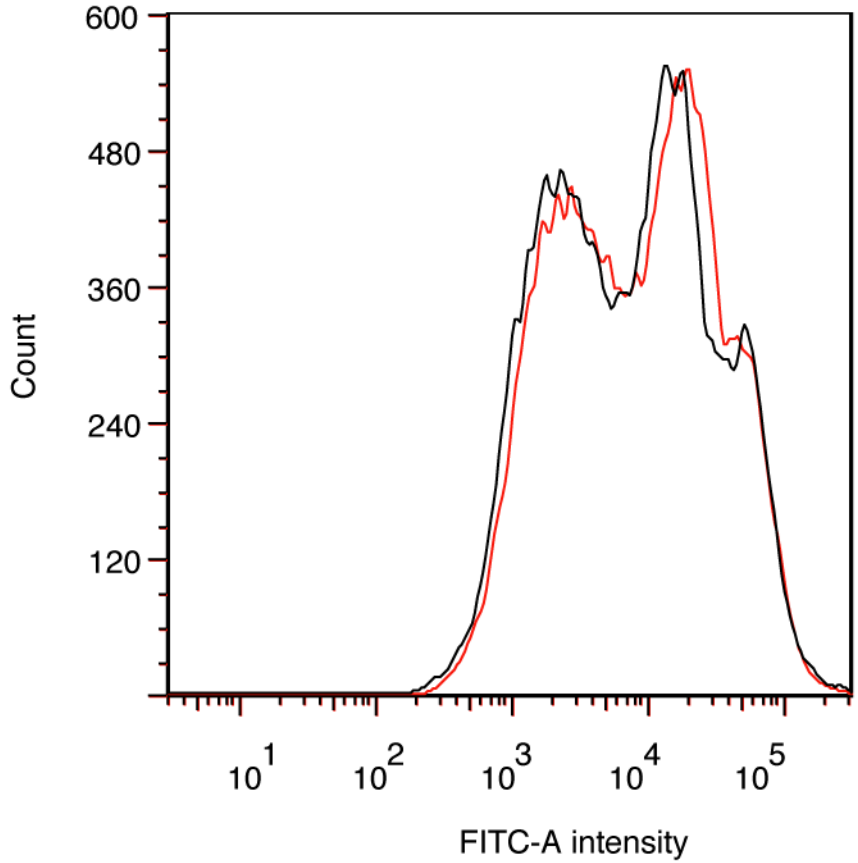
http://pubs.acs.org/subscribe/journals/mdd/v07/i11/html/1104feature_willis.html

FACS transfection analysis



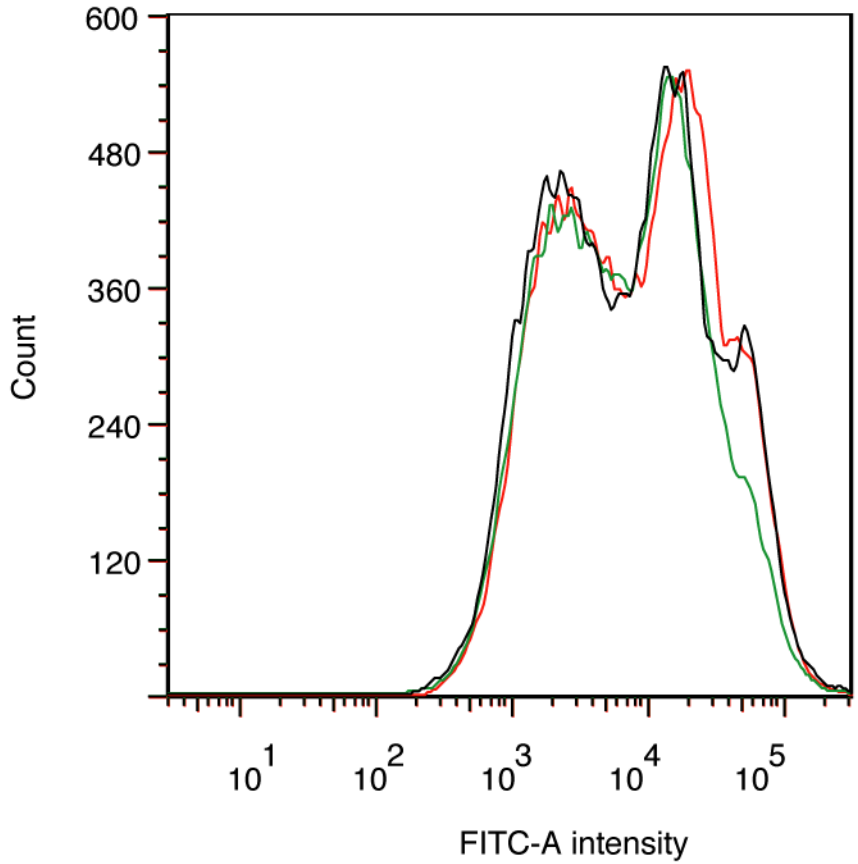
FACS transfection analysis

black - original population
red - non specific siRNA
green - GFP siRNA
purple - HiPerfect transfection reagent



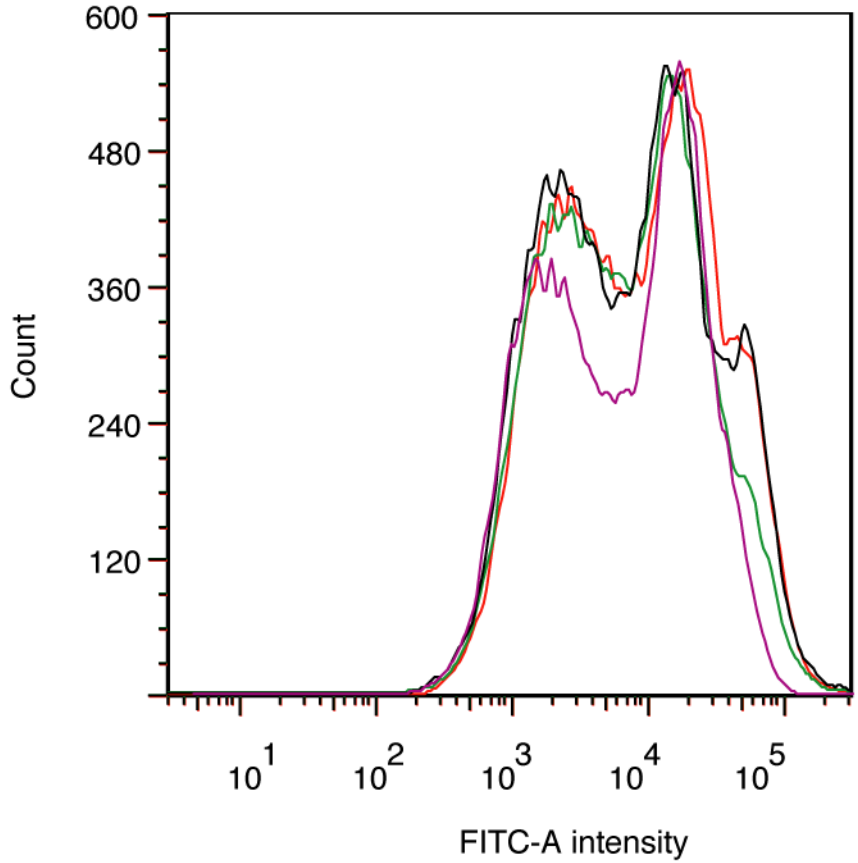
FACS transfection analysis

black - original population
red - non specific siRNA
green - GFP siRNA
purple - HiPerfect transfection reagent



FACS transfection analysis

black - original population
red - non specific siRNA
green - GFP siRNA
purple - HiPerfect transfection reagent



Conclusion

Scaled ultrafast laser cell transfection to biologically-relevant numbers using large area plasmonic substrates.

Demonstrated sequence-specific knockdown of GFP expression in cells.

Further quantitation of cell viability and transfection efficiency must be performed.

Collaborators:

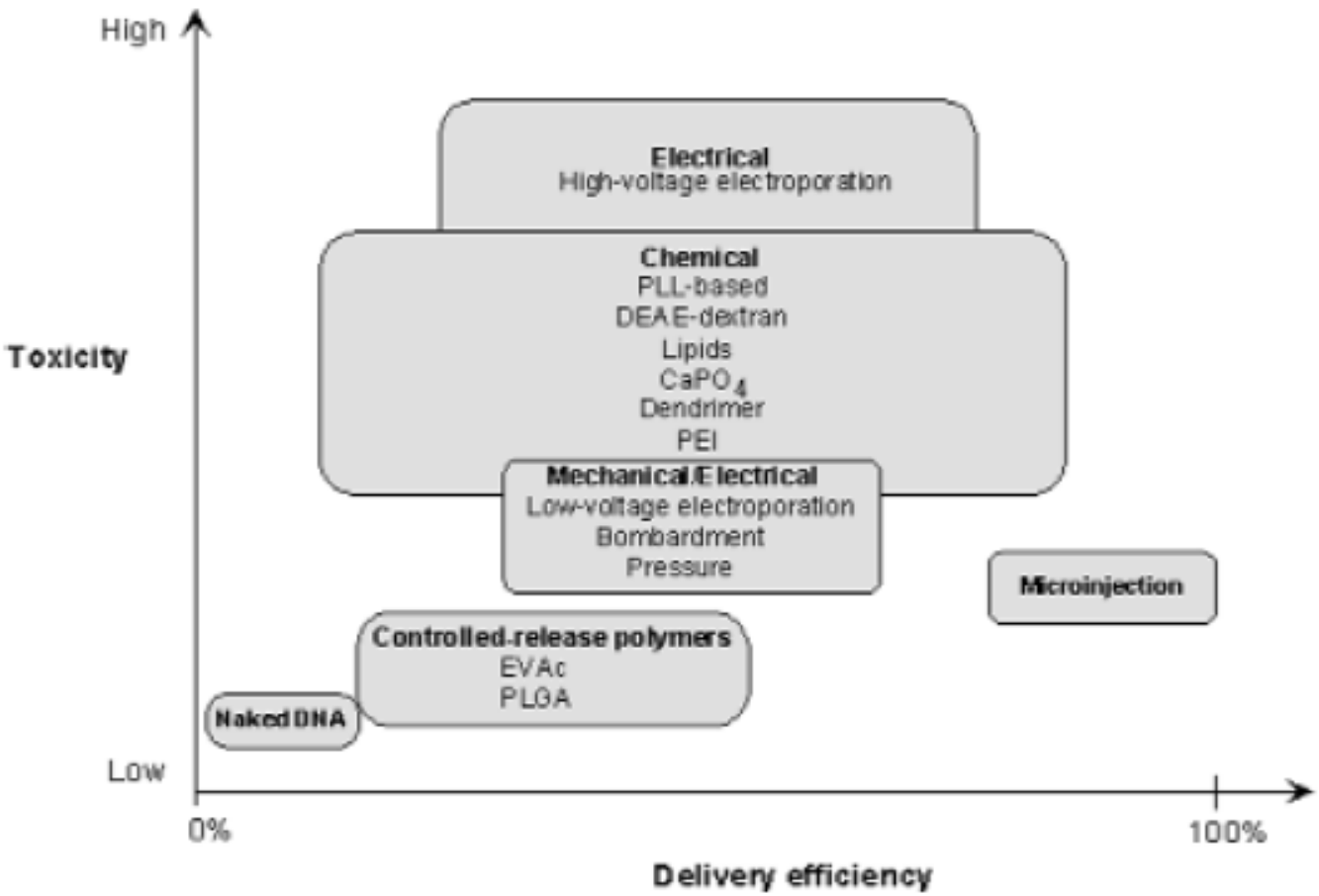
Andrew Koh (Stanford University School of Medicine)
Paul Peng
Valeria Nuzzo
Alex Heisterkamp (Laser Zentrum Hannover)
Eric Mazur

Center for Nanoscale Systems, Harvard University

Questions, comments, ideas?

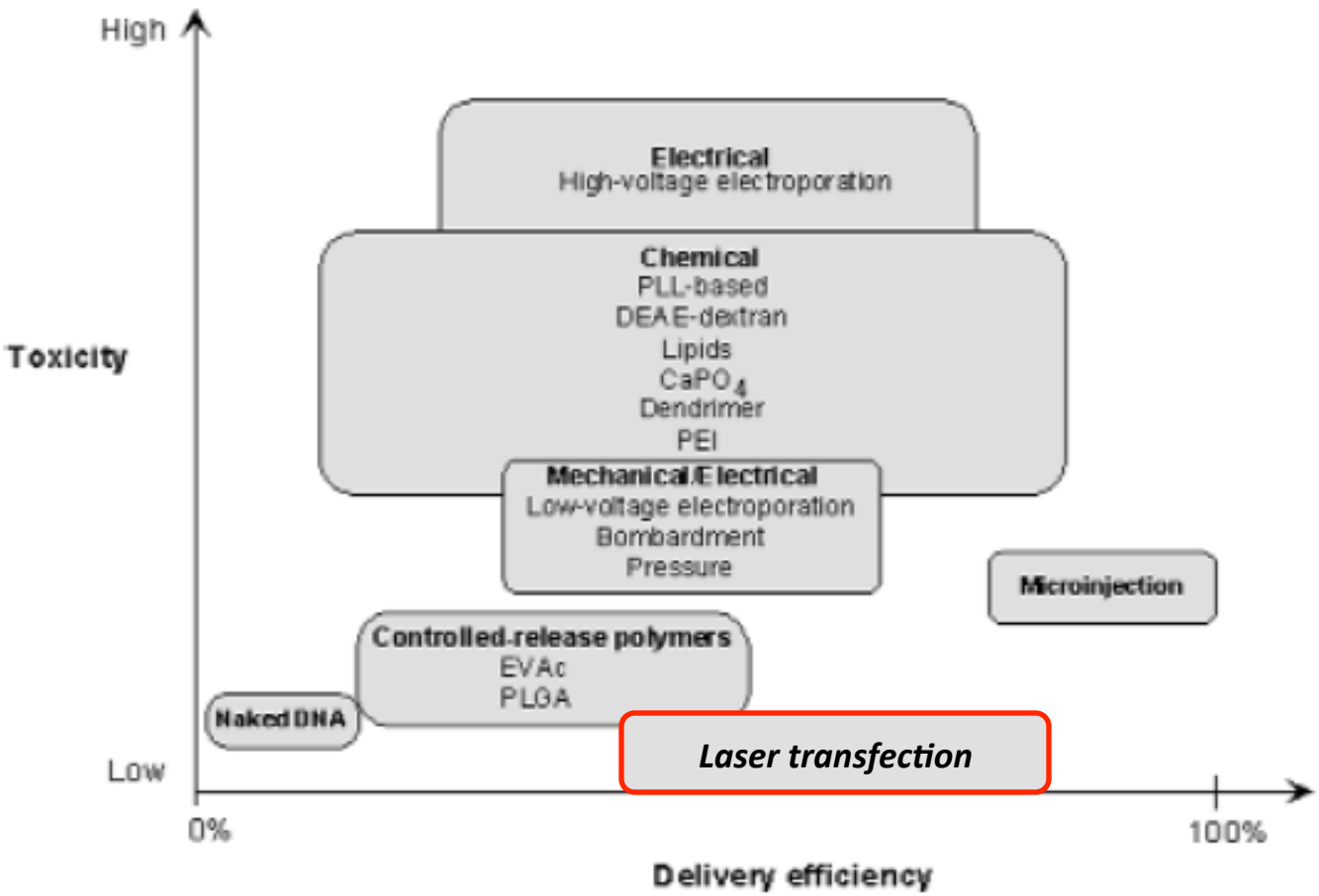
Email: ediebold@post.harvard.edu

Background: femtosecond laser cell transfection



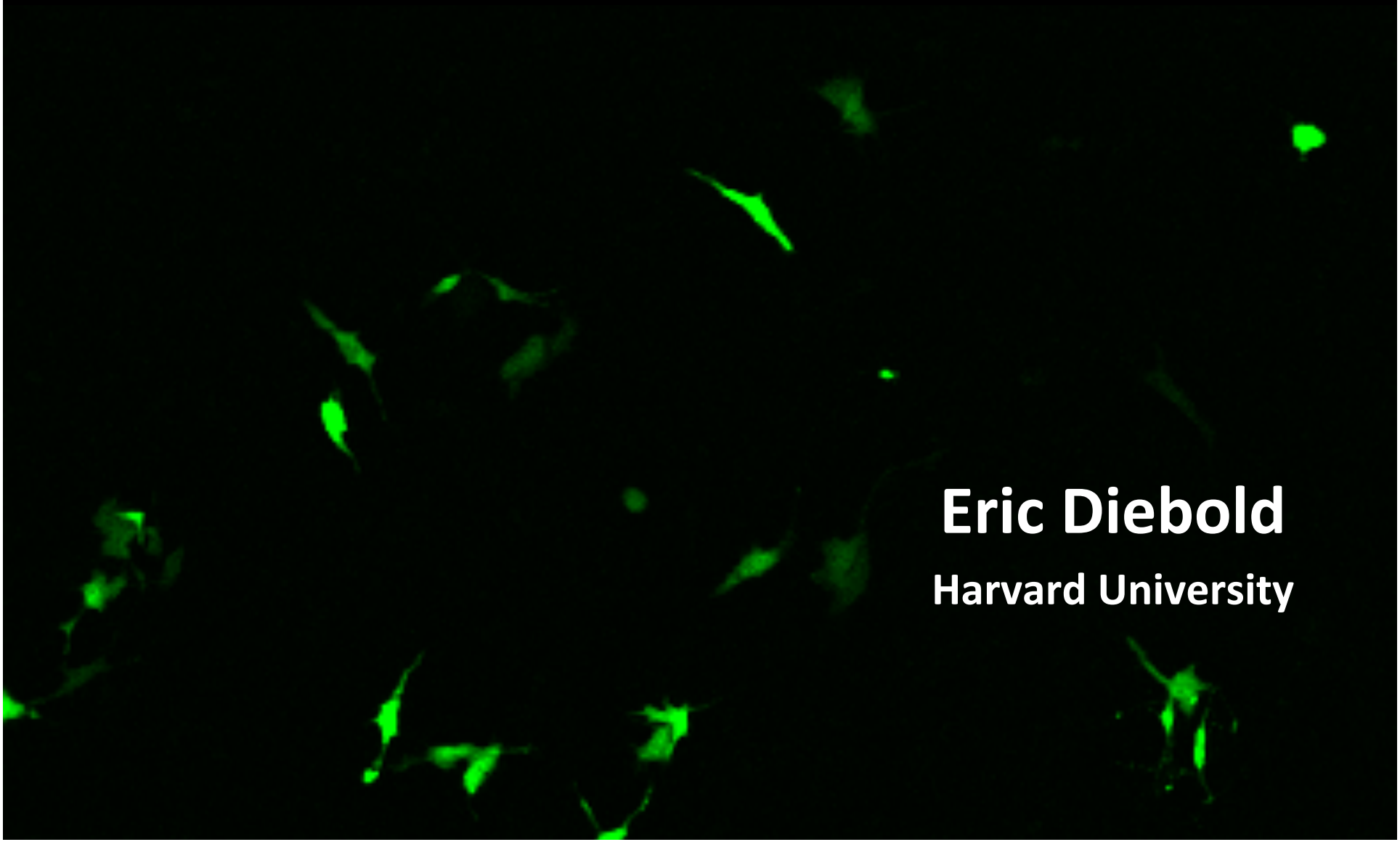
Luo, D. et al. *Nature Biotechnology* **18**, 33 (2000)

Background: femtosecond laser cell transfection



Luo, D. et al. *Nature Biotechnology* **18**, 33 (2000)

Plasmon-enhanced ultrafast laser cell transfection



Eric Diebold

Harvard University