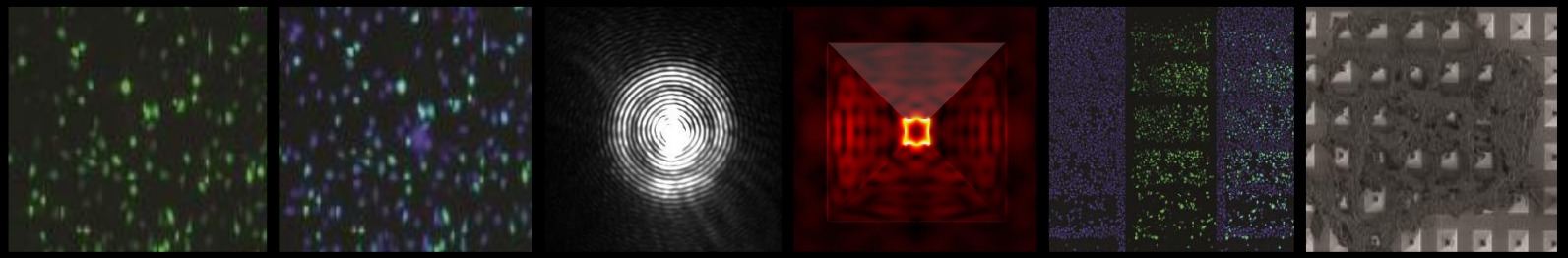


High Throughput Poration of Mammalian Cells using Femtosecond Laser-activated Plasmonic Substrates

Nabiha Saklayen

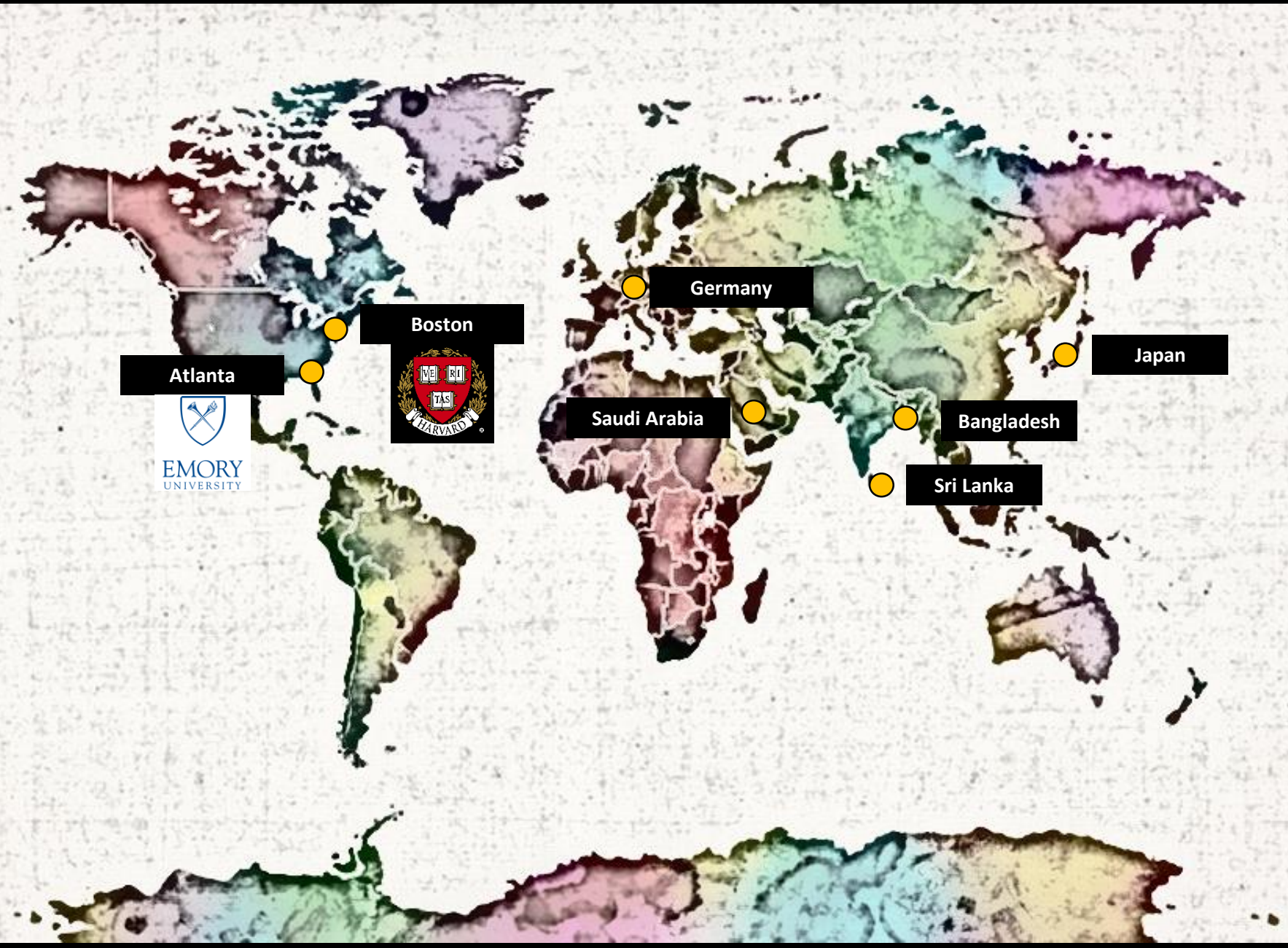
PhD Candidate in Physics

HHMI International Fellow

Mazur Group, Harvard University

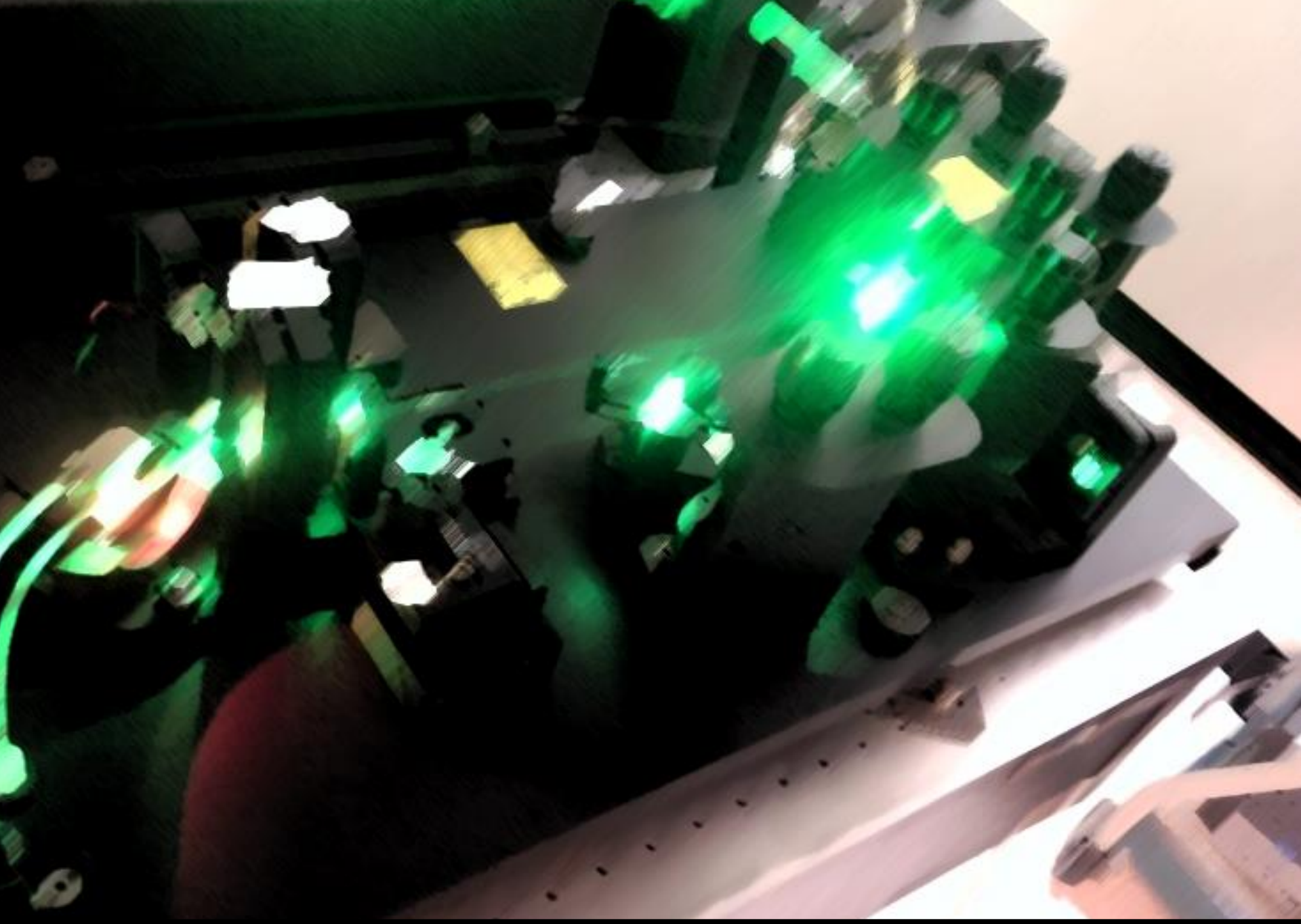
Tokyo Metropolitan University

29 Jan, 2015


Germany
Boston
Atlanta
Japan
Saudi Arabia
Bangladesh
Sri Lanka
EMORY
UNIVERSITY
VE RI TAS
HARVARD

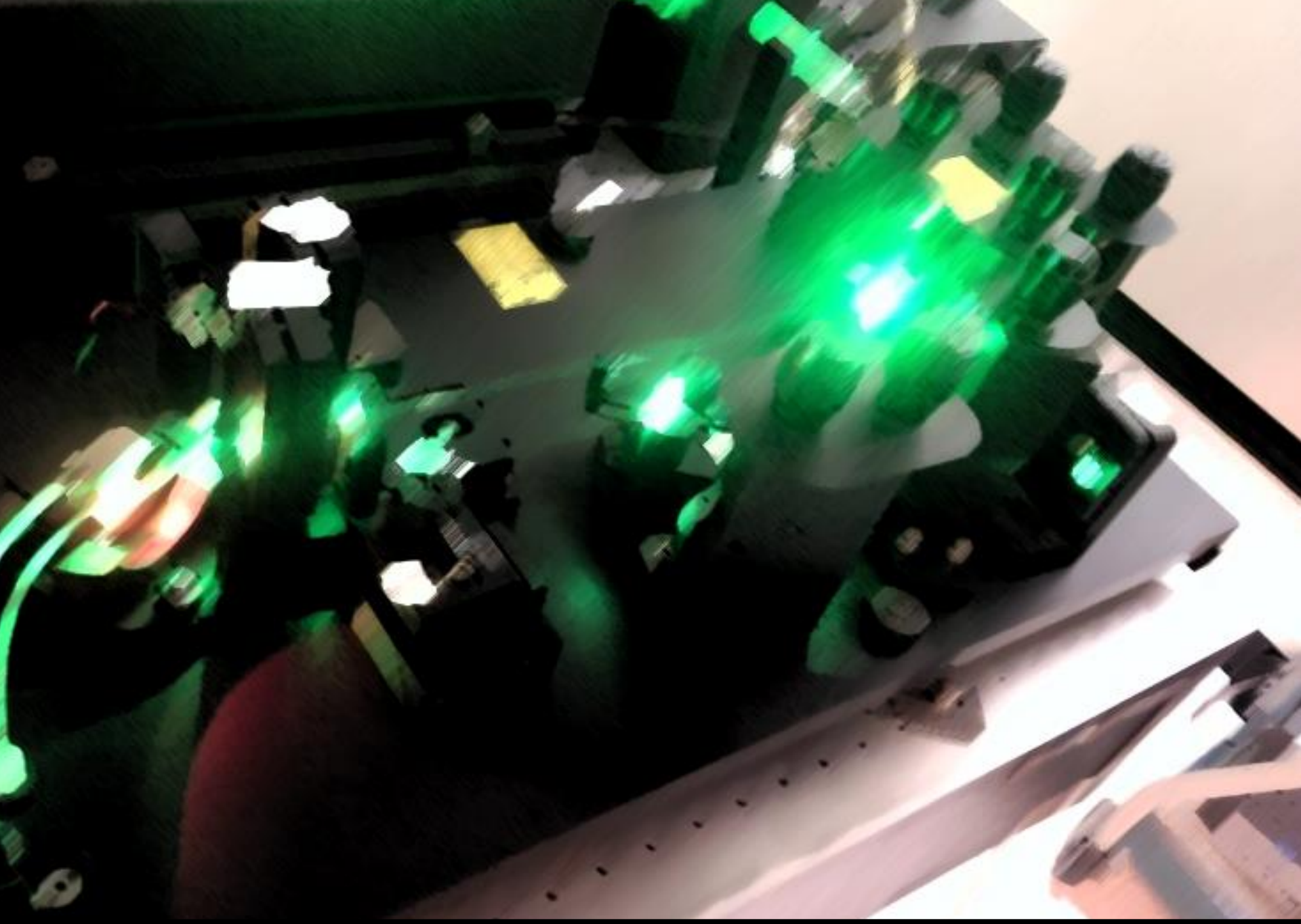

Harvard University
Cambridge, MA

1 Introduction



1 Introduction

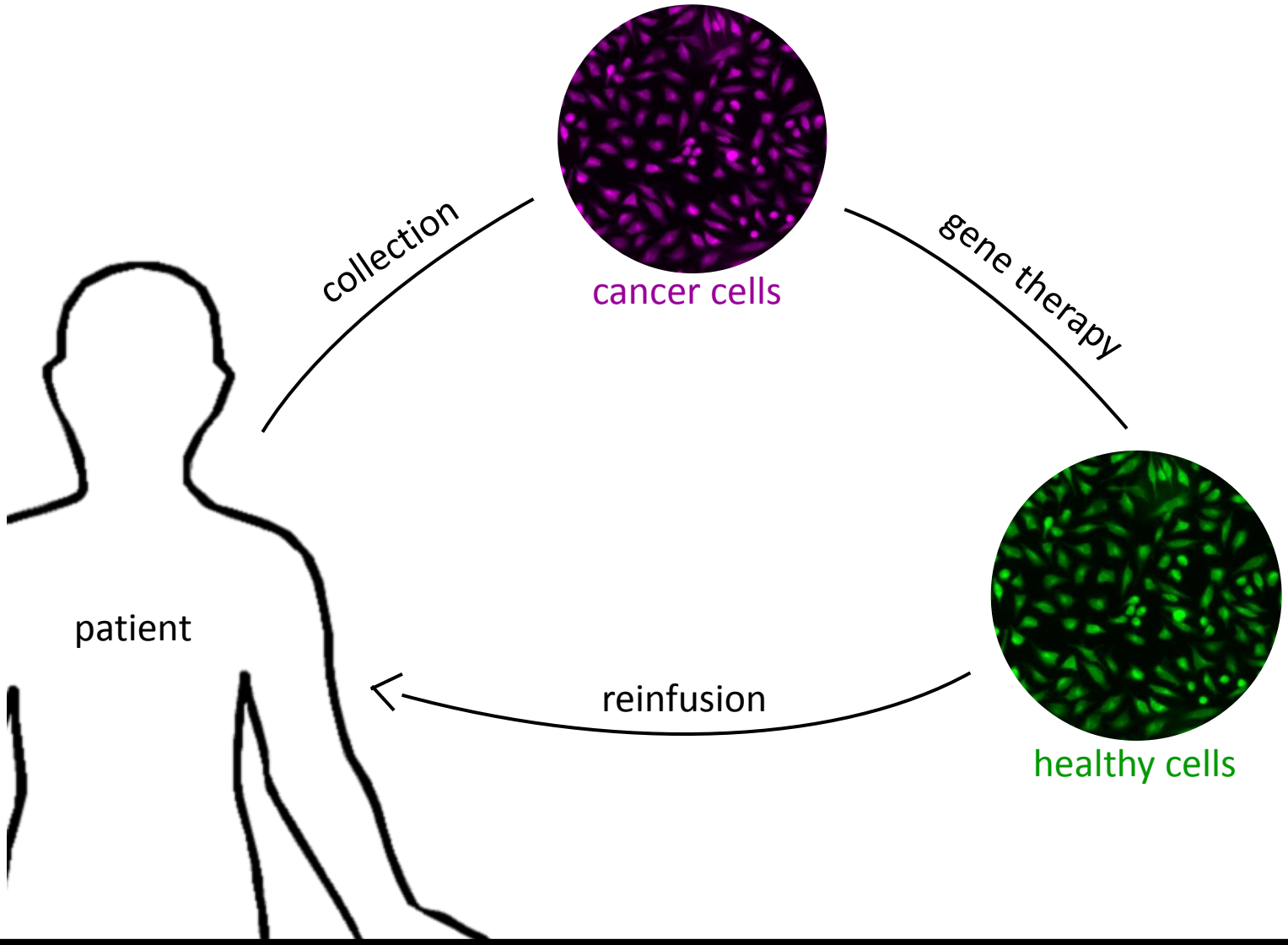
2 Substrates



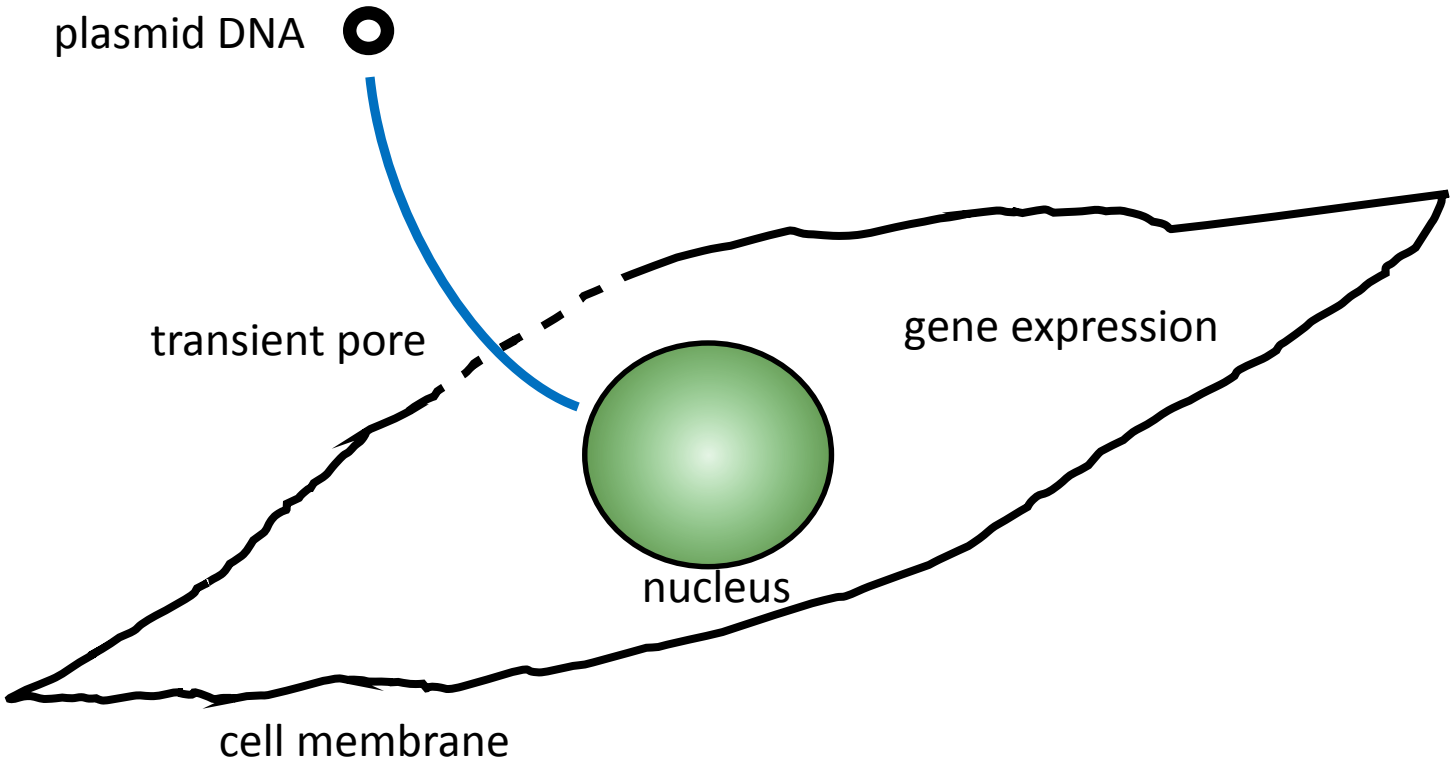
1 Introduction 2 Substrates 3 Experiments

1 Introduction

The future: medicine based on our genes



Transfection introduces genetic vectors into cells for gene expression



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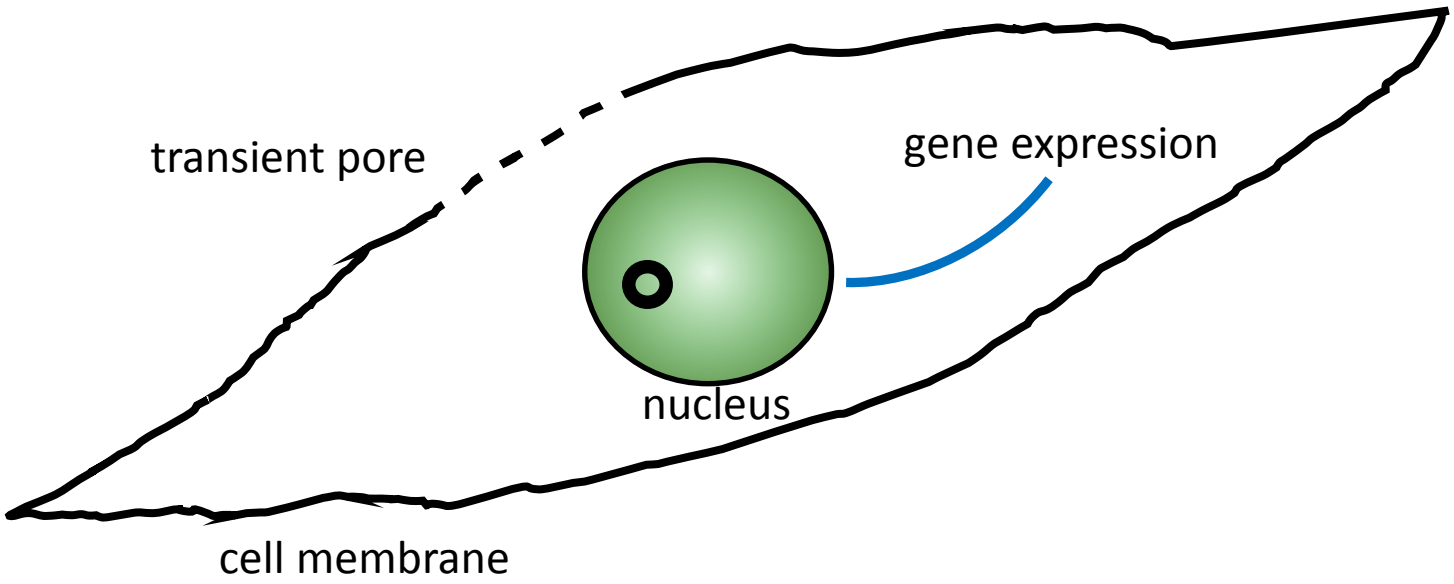


Requirements for a successful transfection platform

	Toxicity	Efficiency	Throughput
Goal	VL	H	H

Synthetic DNA delivery systems *D. Luo et al. Nature Biotechnology (2000)*

Viral transfection is most popular, but comes with immunological risks

	Toxicity	Efficiency	Throughput
Goal	VL	H	H
Viral transfection	M	H	H

Synthetic DNA delivery systems *D. Luo et al. Nature Biotechnology (2000)*

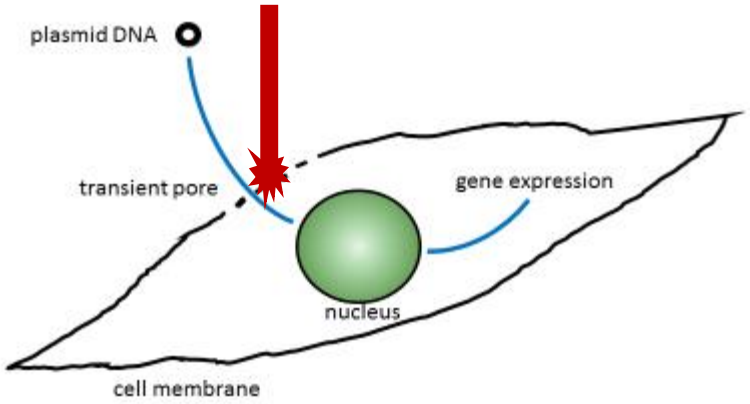
None of the available transfection methods meet all requirements

	Toxicity	Efficiency	Throughput
Goal	VL	H	H
Polymer/Lipid	M	M	H
Electroporation	H	H	H
Naked DNA	VL	L	H
Viral transfection	M	H	H
Optotransfection	L	H	L
Plasmonic NPs	M	H	H

Synthetic DNA delivery systems *D. Luo et al. Nature Biotechnology (2000)*

Example 1: Optotransfection offers high efficiency and low toxicity

	Toxicity	Efficiency	Throughput
Goal	VL	H	H
Optotransfection	VL	H	



Synthetic DNA delivery systems *D. Luo et al. Nature Biotechnology (2000)*

Example 1: Optotransfection offers high efficiency and low toxicity

	Toxicity	Efficiency	Throughput
Goal	VL	H	H
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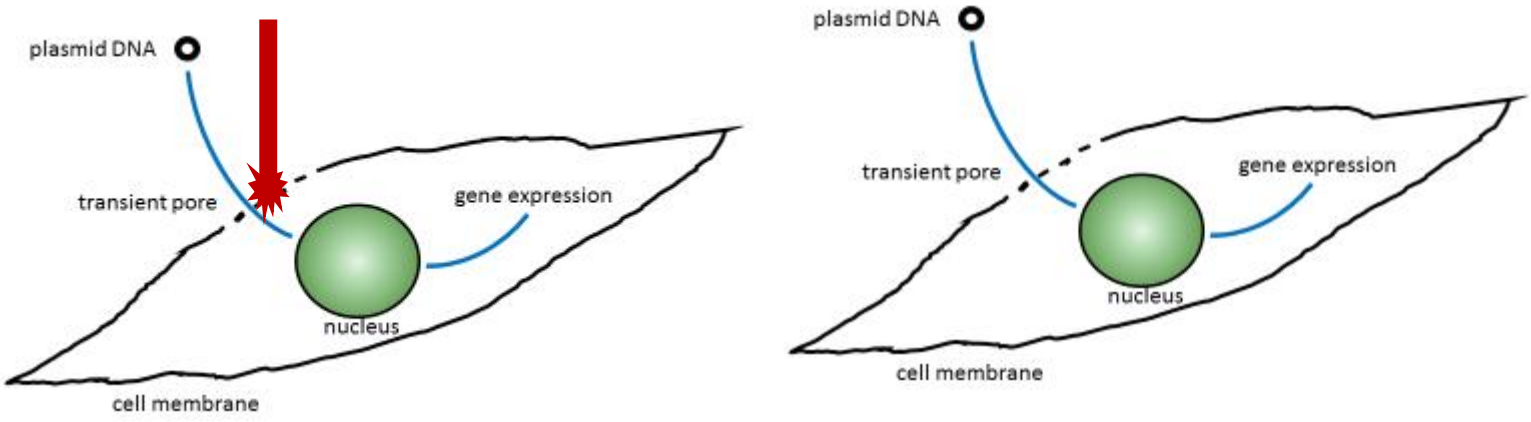
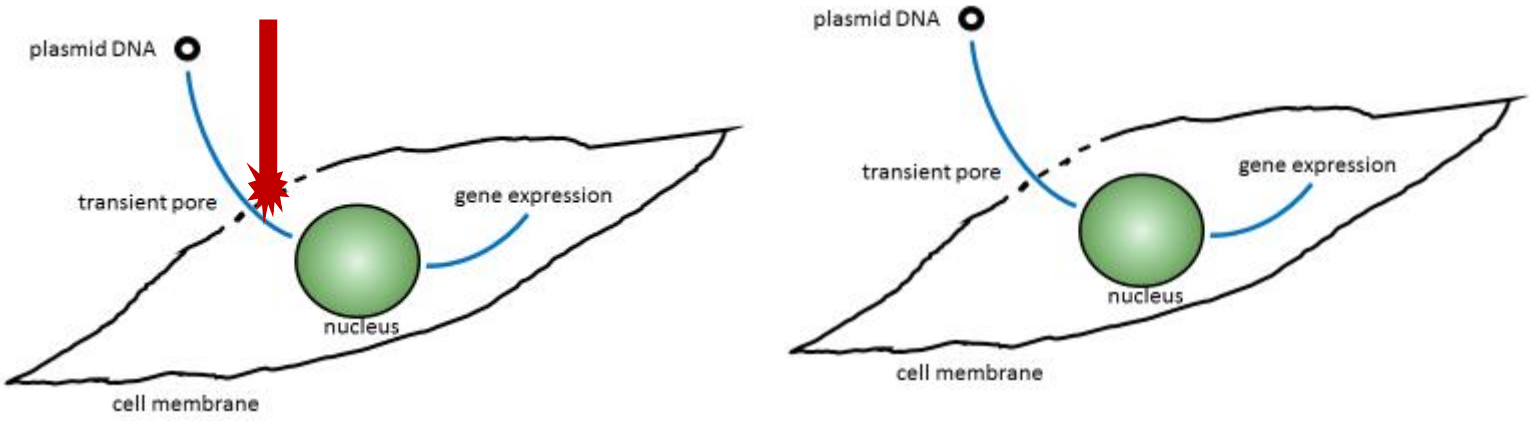


Synthetic DNA delivery systems *D. Luo et al. Nature Biotechnology (2000)*

Example 1: Optotransfection offers ***extremely*** low throughput

	Toxicity	Efficiency	Throughput
Goal	VL	H	H
Optotransfection	VL	H	L



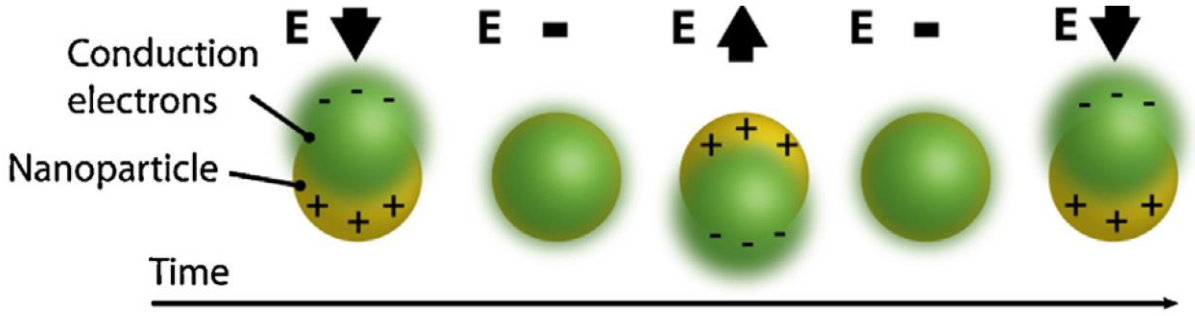
Synthetic DNA delivery systems *D. Luo et al. Nature Biotechnology (2000)*

Example 2: Plasmonic nanoparticle transfection offers high throughput and high efficiency

	Toxicity	Efficiency	Throughput
Goal	VL	H	H
Plasmonic NPs	M	H	H

Example 2: Plasmonic nanoparticle transfection uses Localized Surface Plasmons (LSPs)

	Toxicity	Efficiency	Throughput
Goal	VL	H	H
Plasmonic NPs	M	H	H



Plasmonics for pulsed-laser cell surgery: Fundamentals and applications *E. Boulais et al. Journal of Photochemisty and Photobiology C: Photochemistry Reviews 2013*

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	Toxicity	Efficiency	Throughput
Goal	VL	H	H
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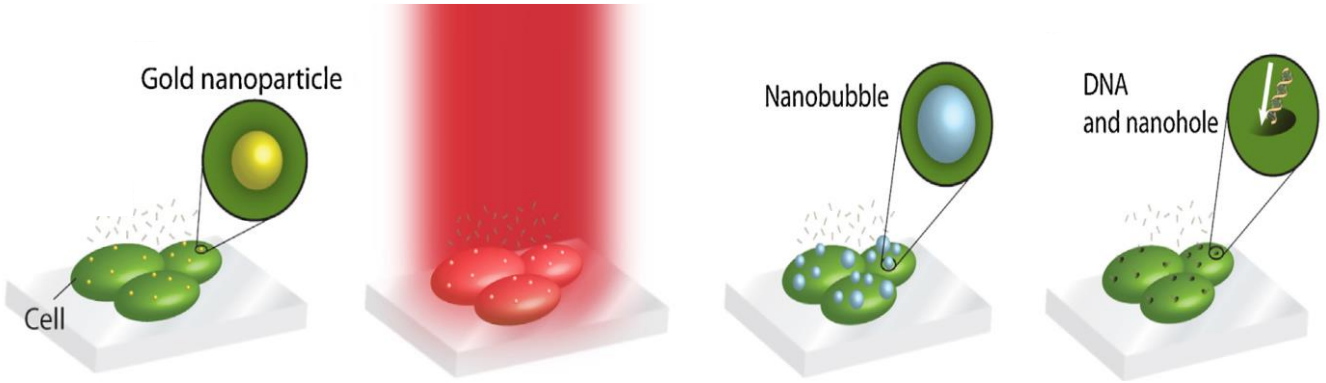

Plasmonics for pulsed-laser cell surgery: Fundamentals and applications *E. Boulais et al. Journal of Photochemisty and Photobiology C: Photochemistry Reviews 2013*

Example 2: Plasmonic nanoparticle transfection comes with ***toxicity*** from ***particle residue***

	Toxicity	Efficiency	Throughput
Goal	VL	H	H
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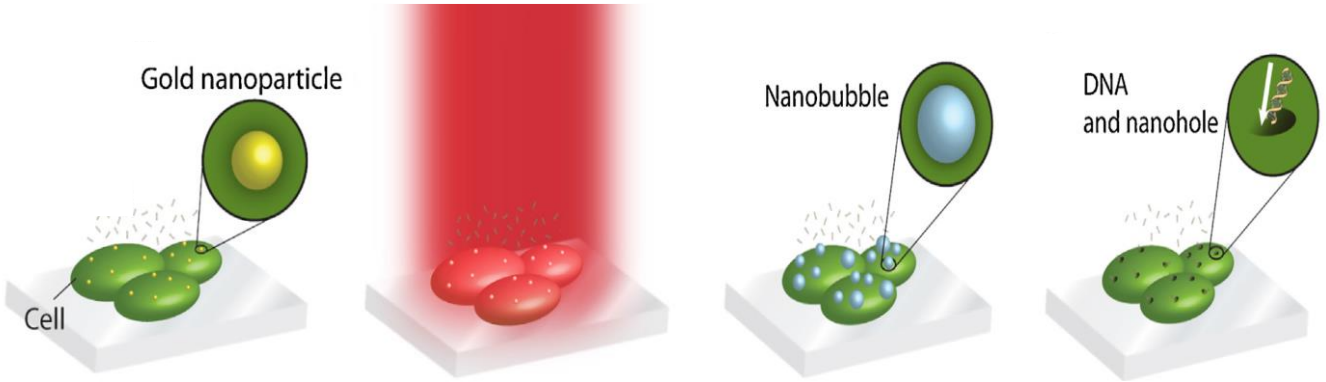
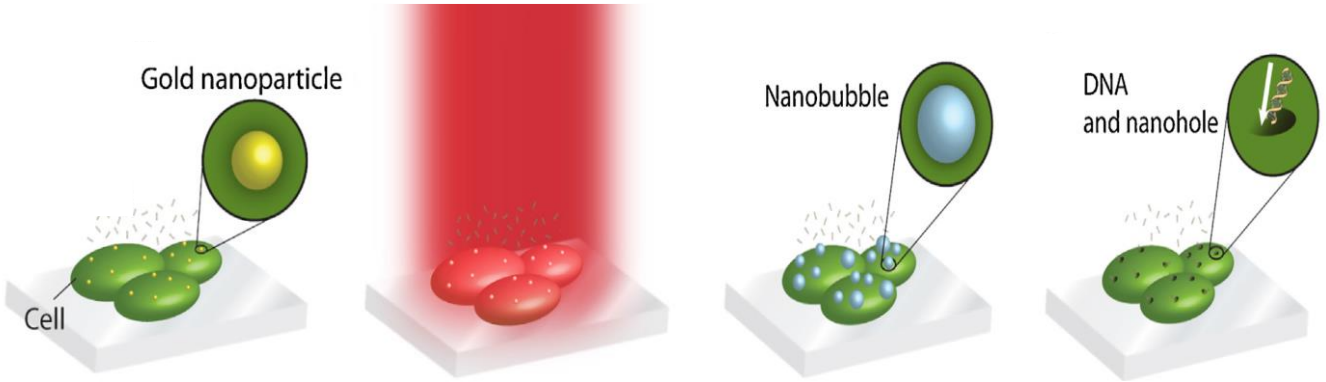


Plasmonics for pulsed-laser cell surgery: Fundamentals and applications *E. Boulais et al. Journal of Photochemisty and Photobiology C: Photochemistry Reviews 2013*

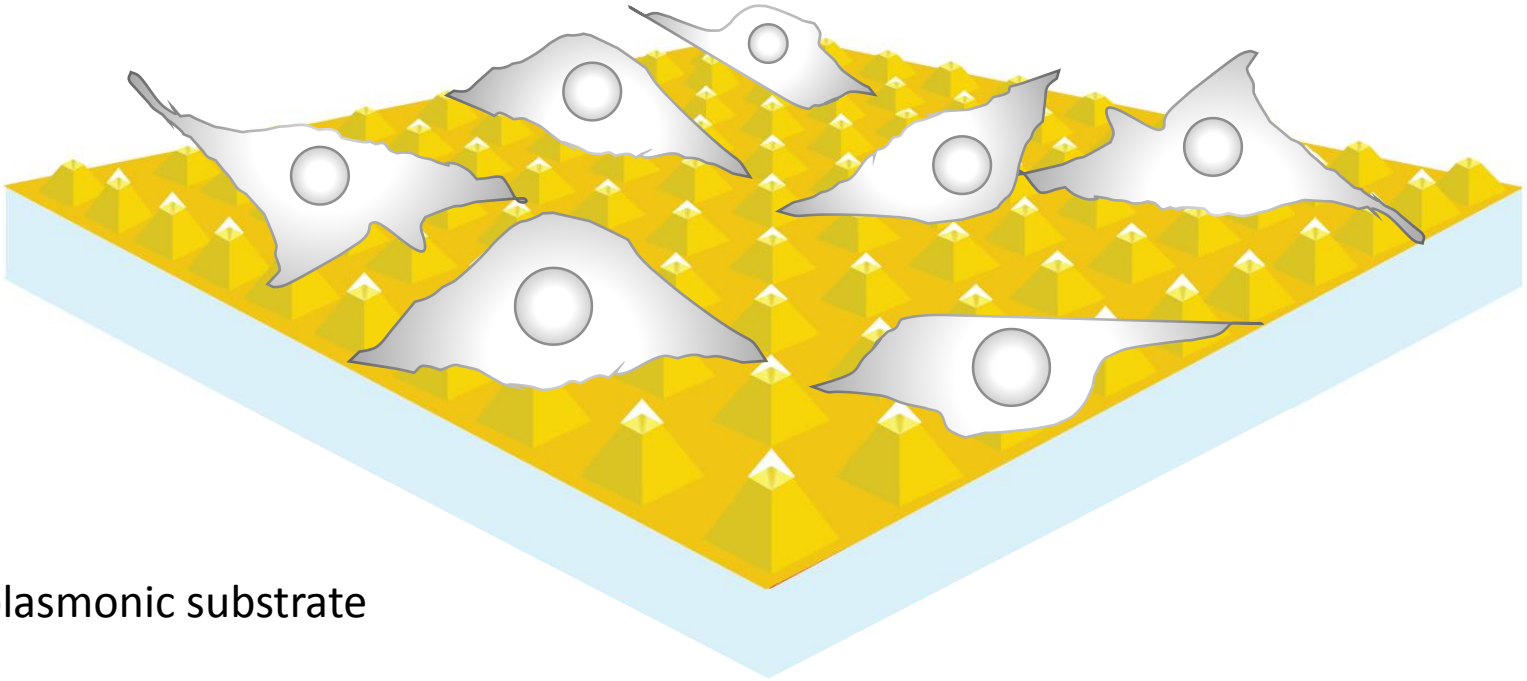
Example 2: Plasmonic nanoparticle transfection comes with ***toxicity*** from ***particle residue***

	Toxicity	Efficiency	Throughput
Goal	VL	H	H

Need for a new transfection method

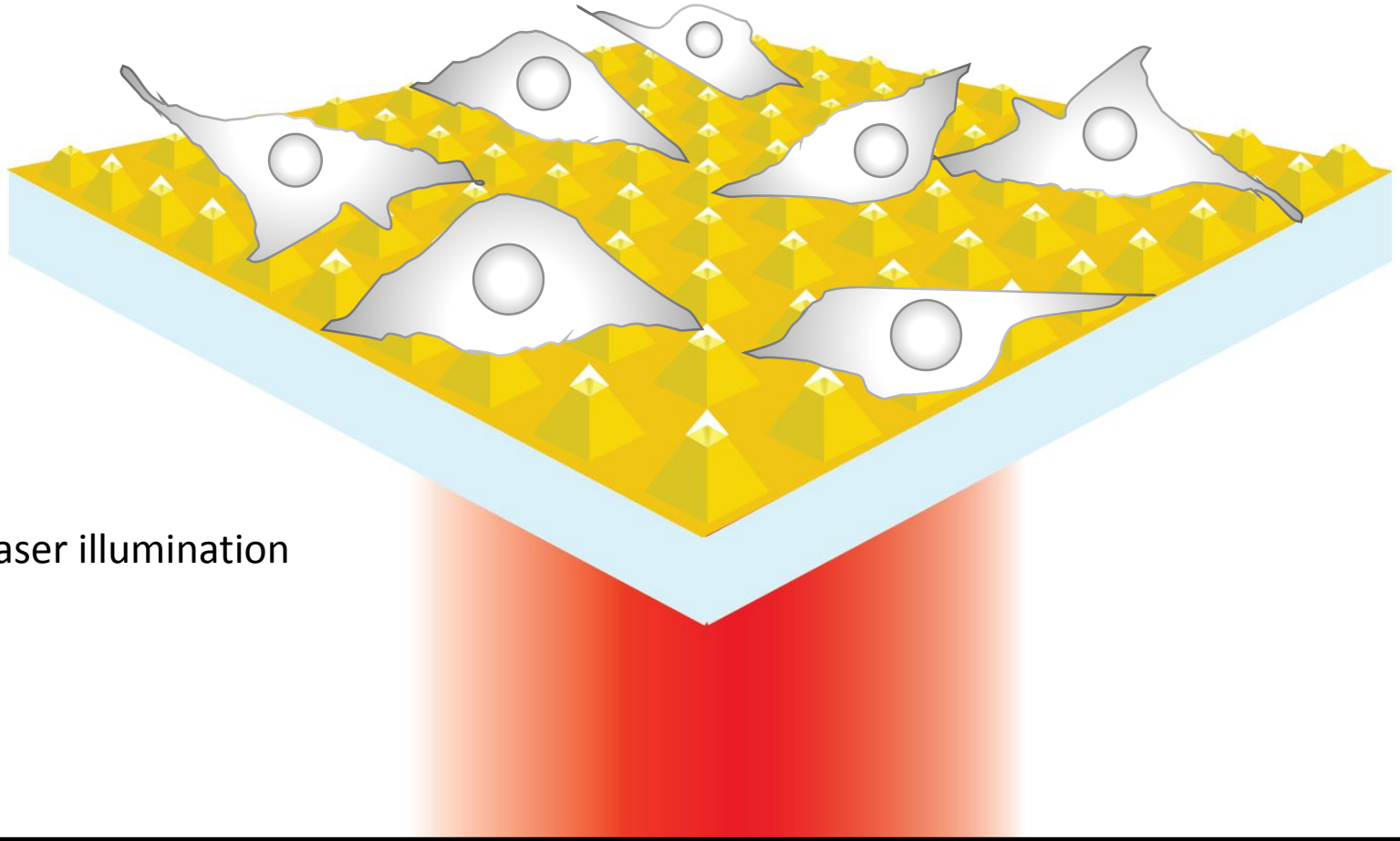


New approach: plasmonic pyramid substrates

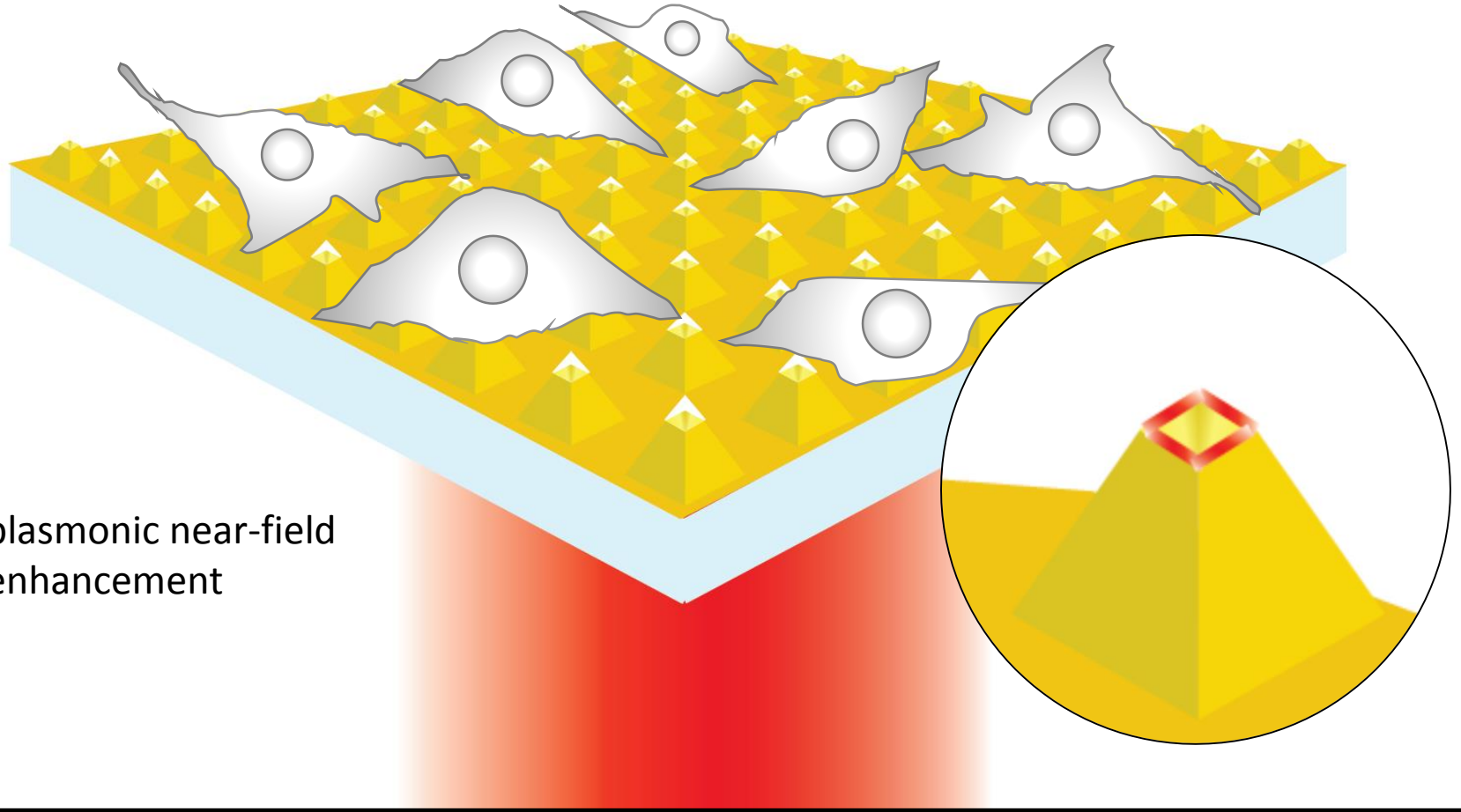


plasmonic substrate

New approach: plasmonic pyramid substrates

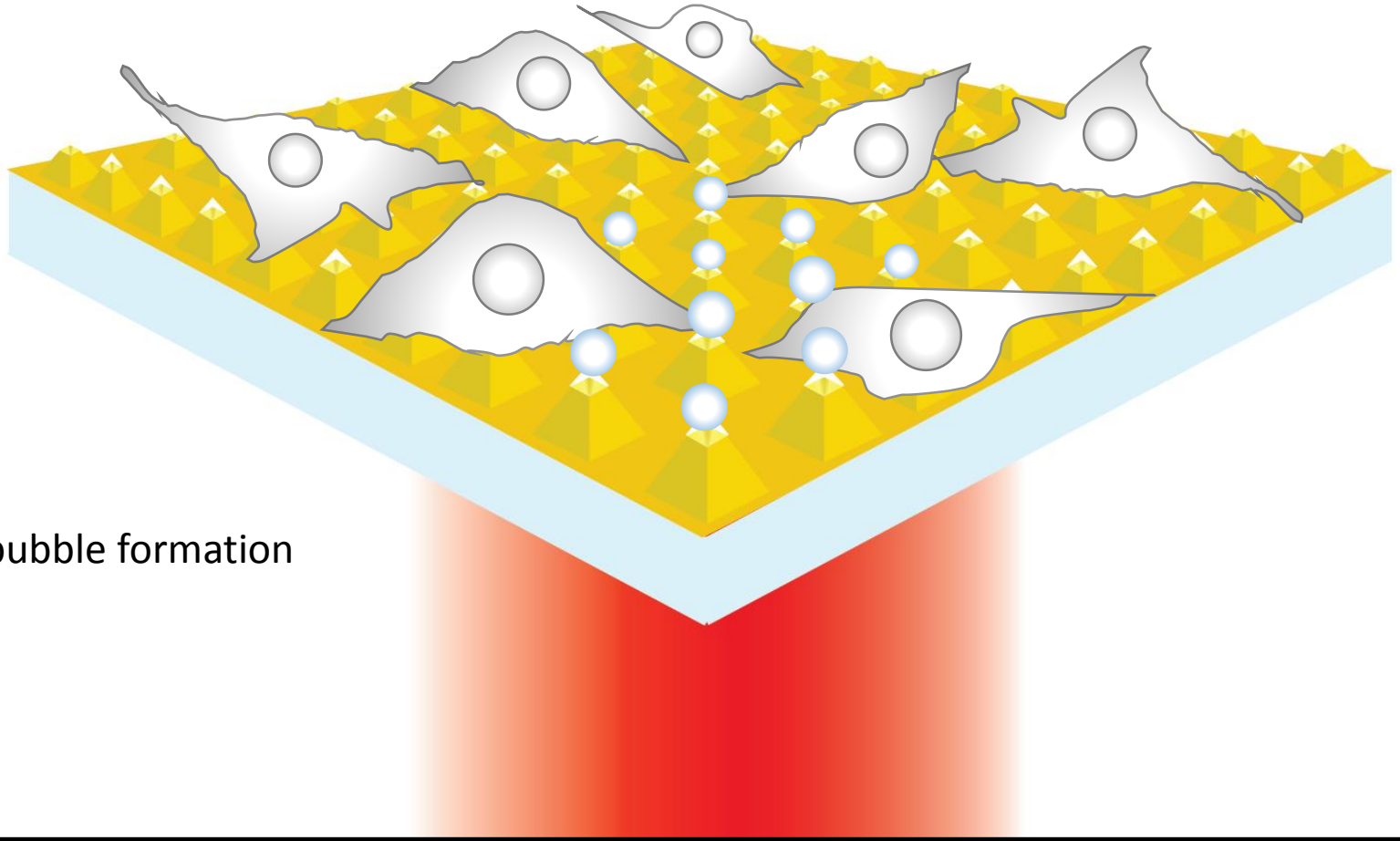


New approach: plasmonic pyramid substrates



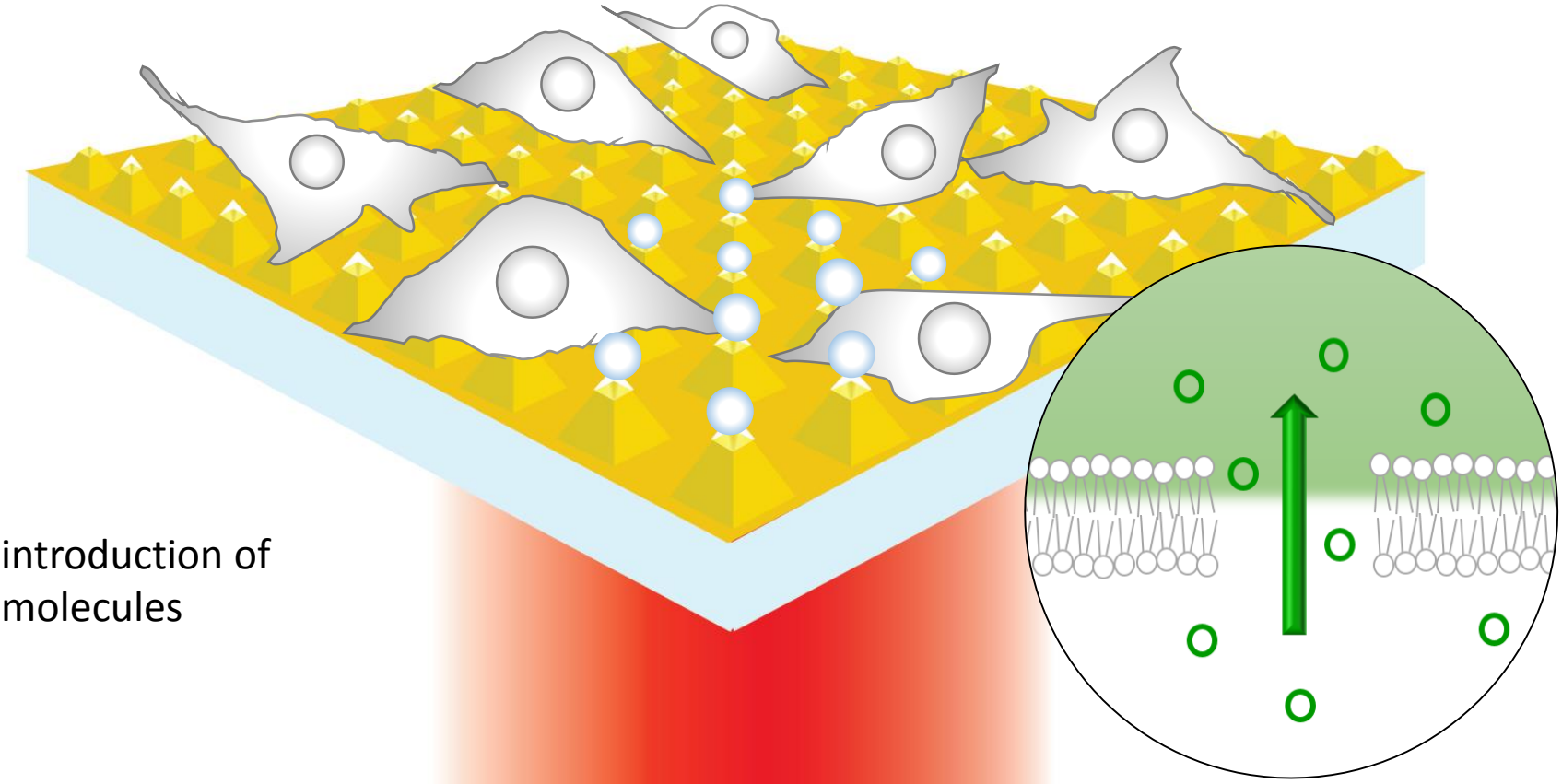
plasmonic near-field enhancement

New approach: plasmonic pyramid substrates



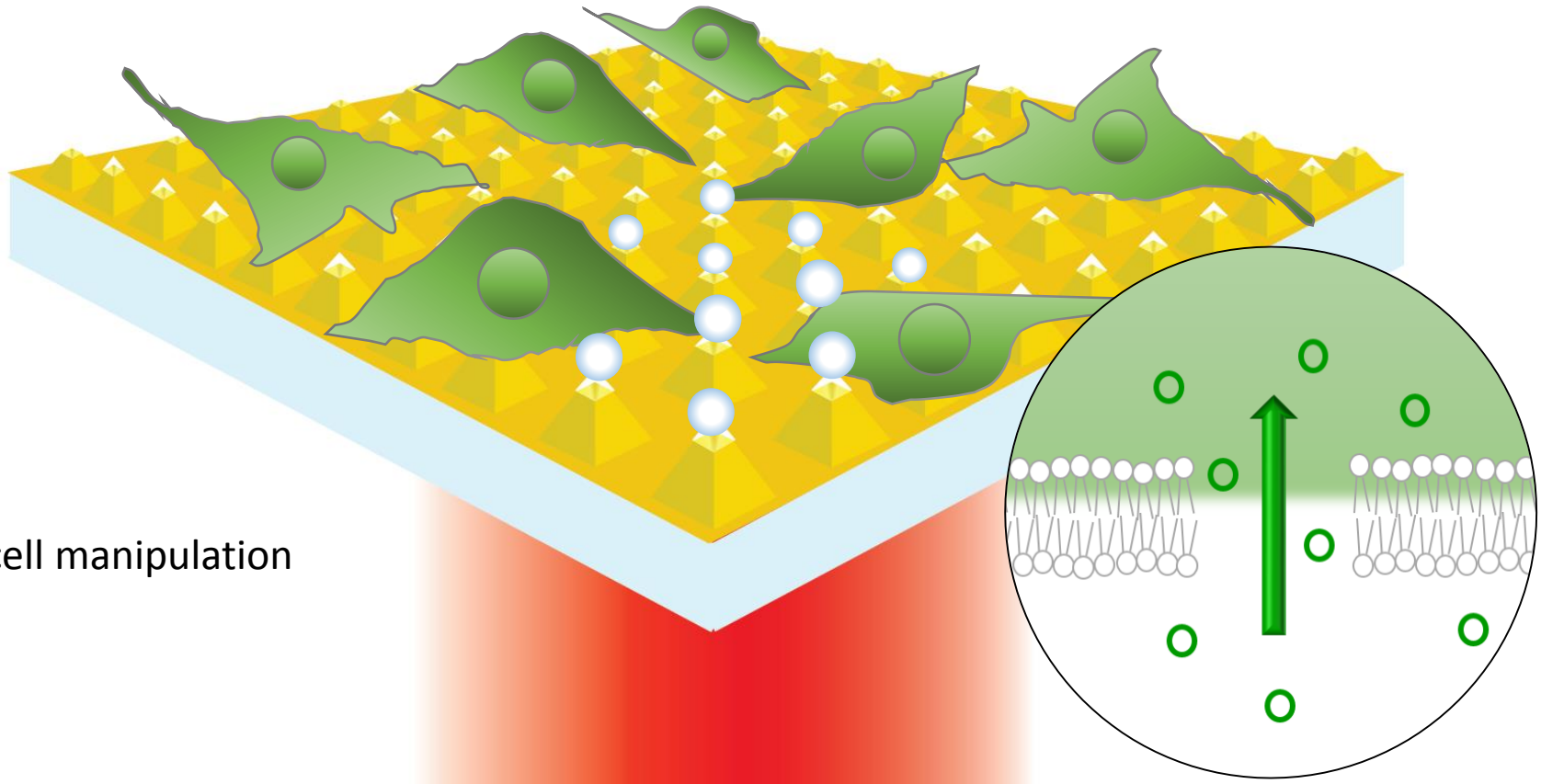
bubble formation

New approach: plasmonic pyramid substrates



introduction of molecules

New approach: plasmonic pyramid substrates



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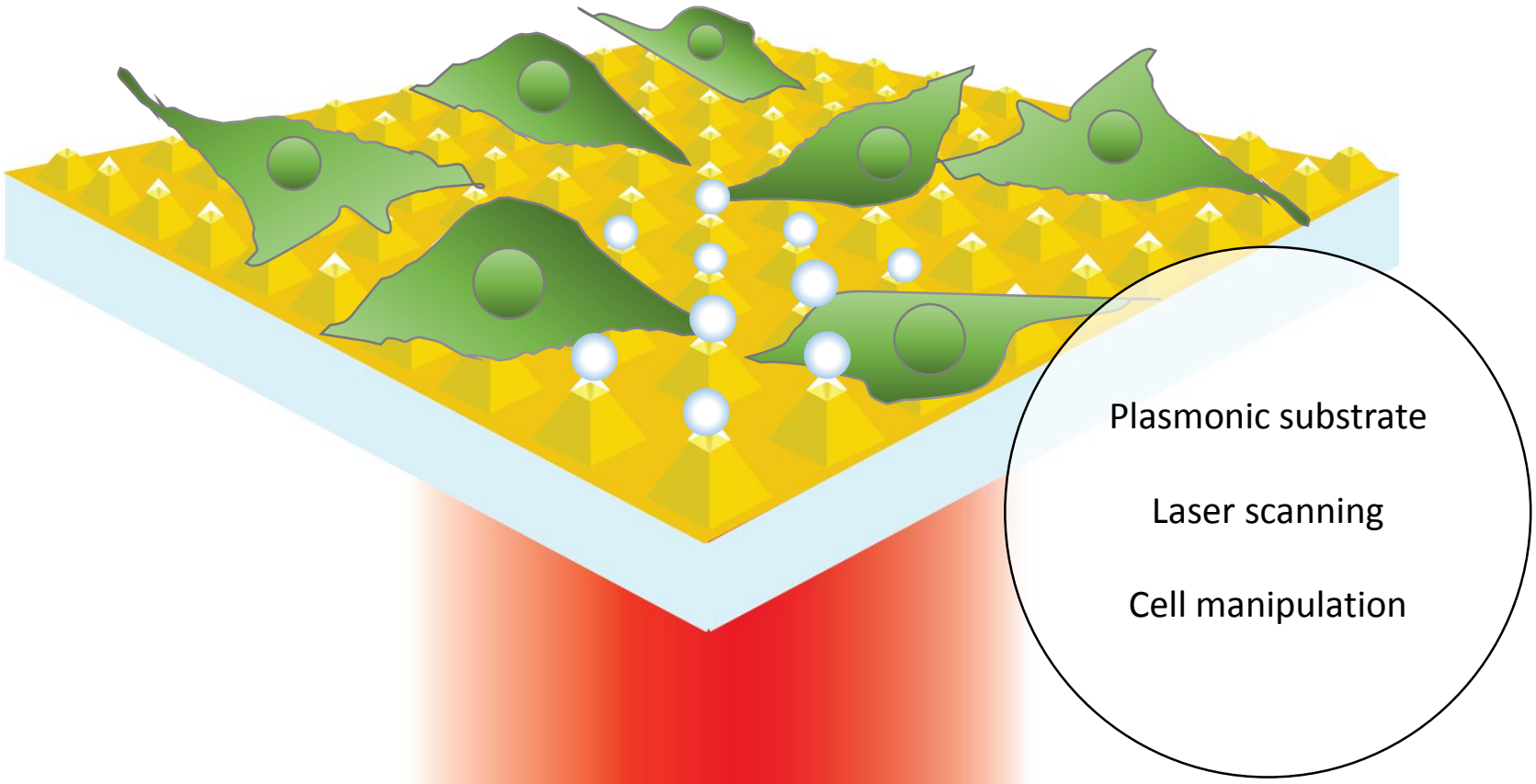
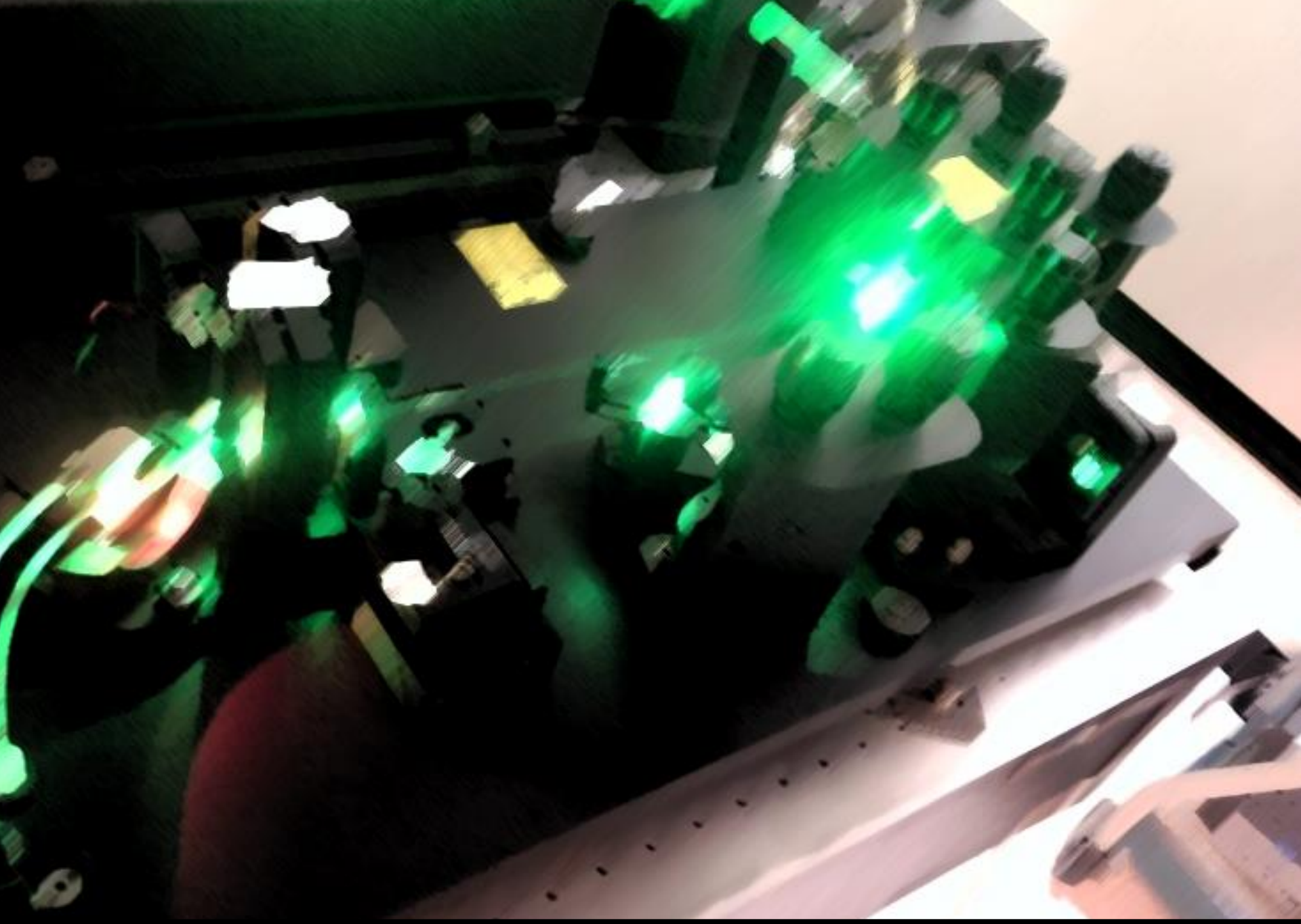
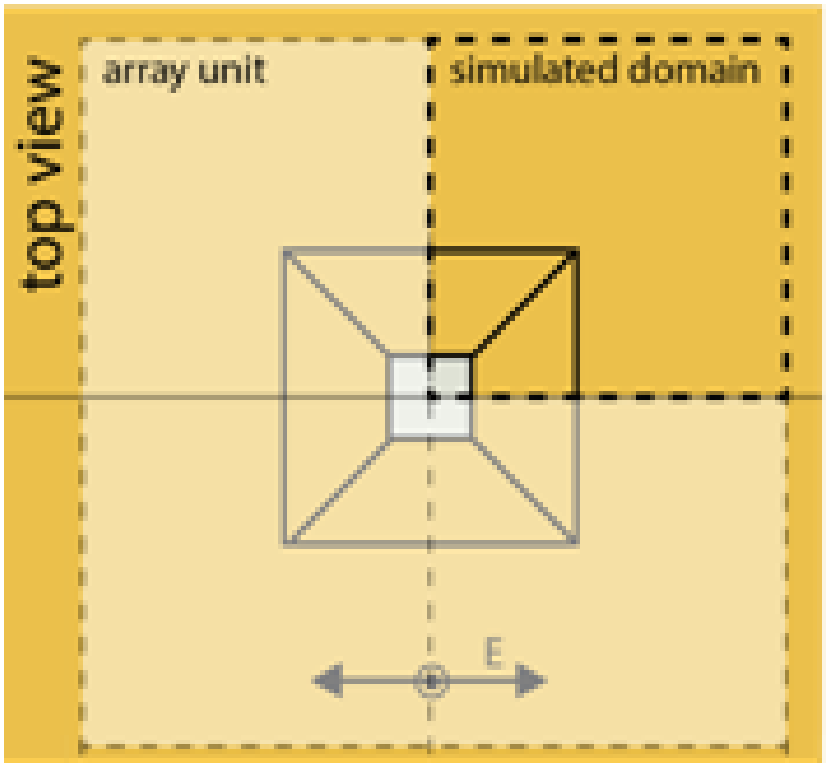


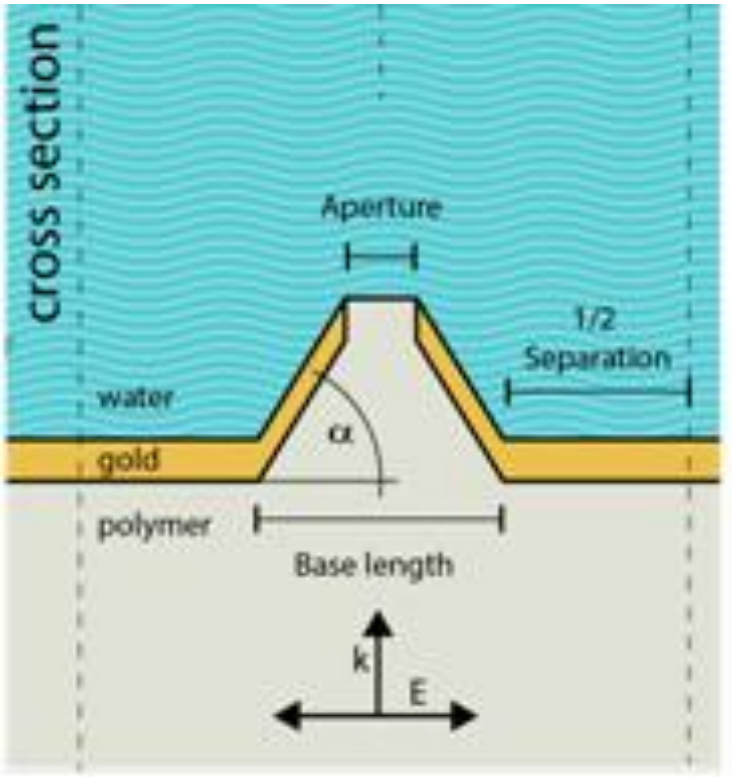


1 Introduction

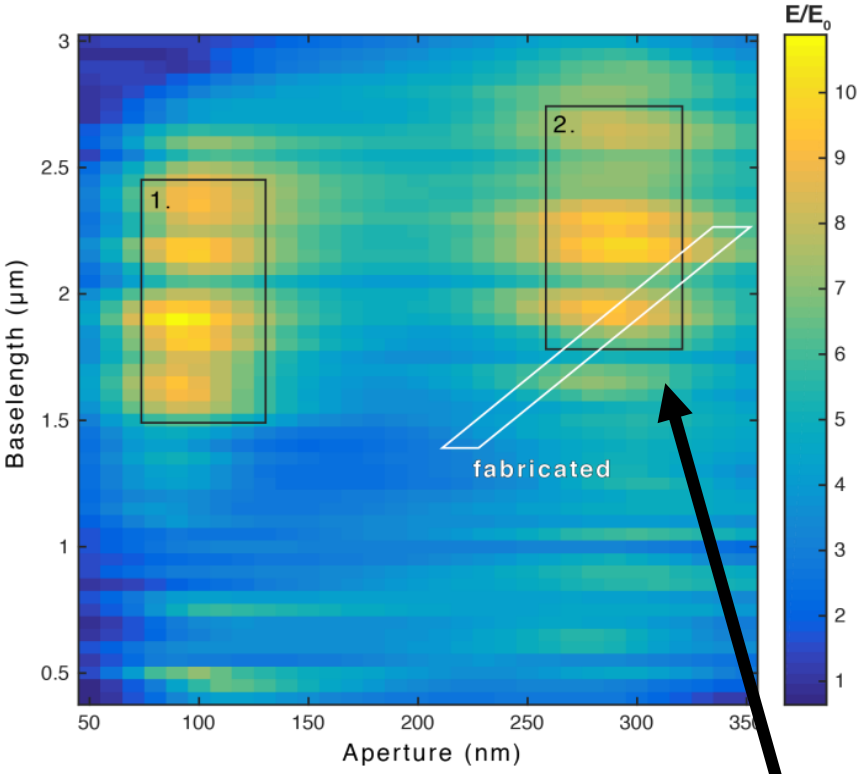
2 Substrates

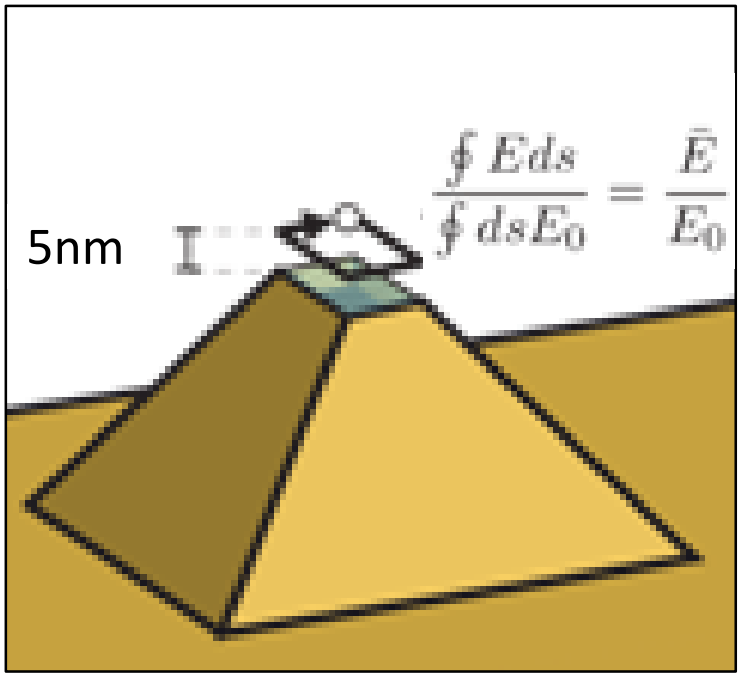
Our micropyramids have nano-apertures on top





Simulations tell us the geometrical parameters for highest near-field enhancement

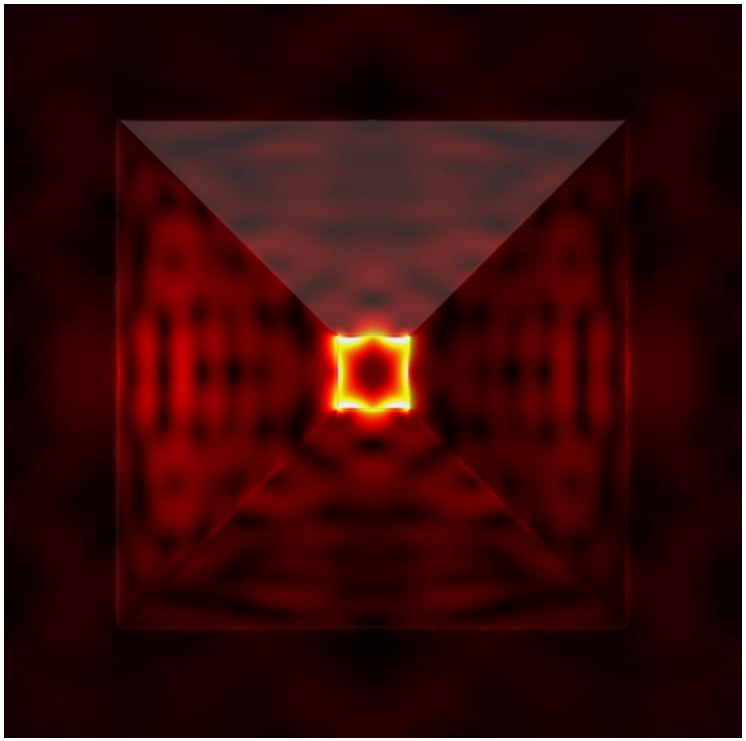




high near-field enhancement

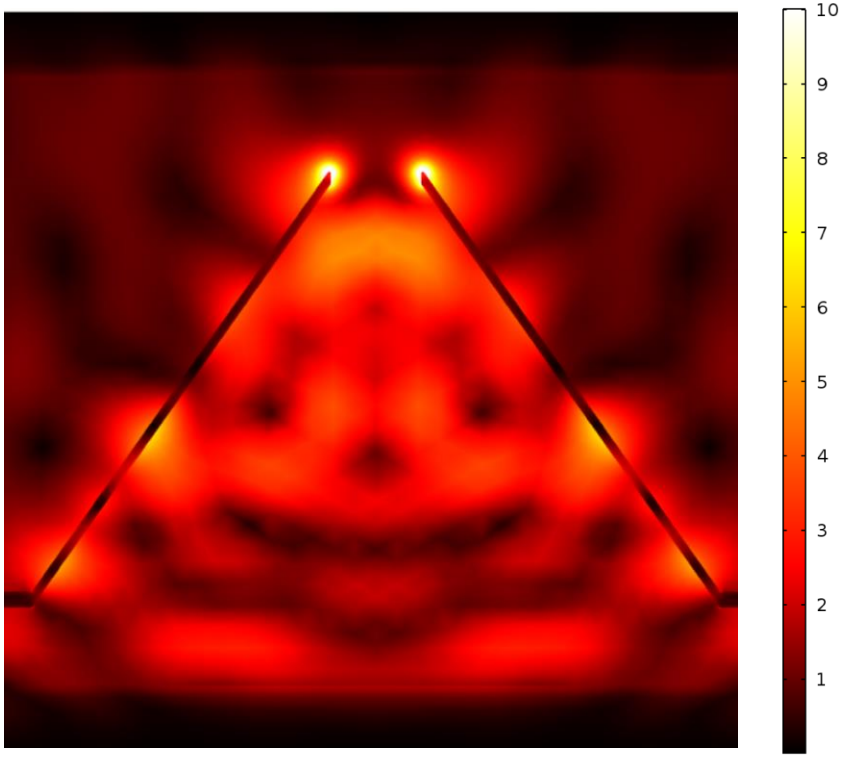
Simulations tell us the geometrical parameters for highest near-field enhancement

Top view



Baselength: 2200nm
Seperation: 1000nm

Cross section



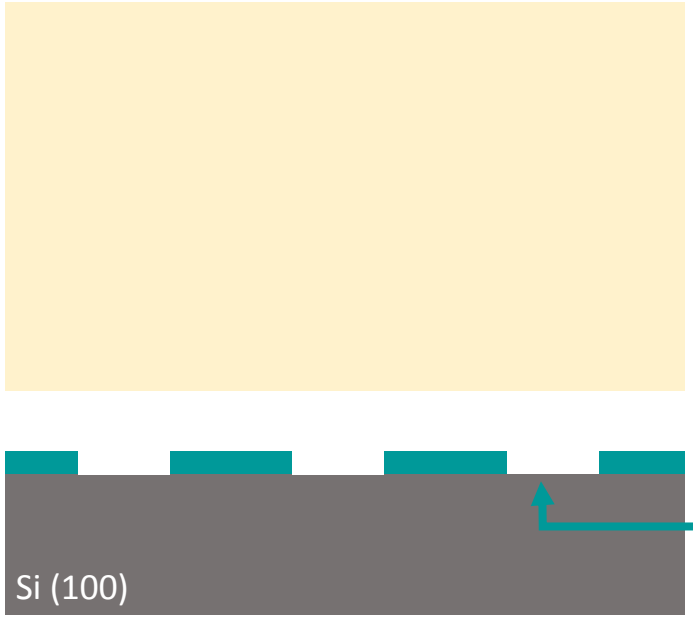
Aperture: 290nm
Gold Thickness: 50nm

Pyramids fabricated at Harvard Center for Nanoscale Science

Photolithography is used to fabricate large arrays of micropyramids

Photolithography is used to fabricate large arrays of micropyramids

Photolithography



Photolithography is used to fabricate large arrays of micropyramids

KOH
Anisotropic etching

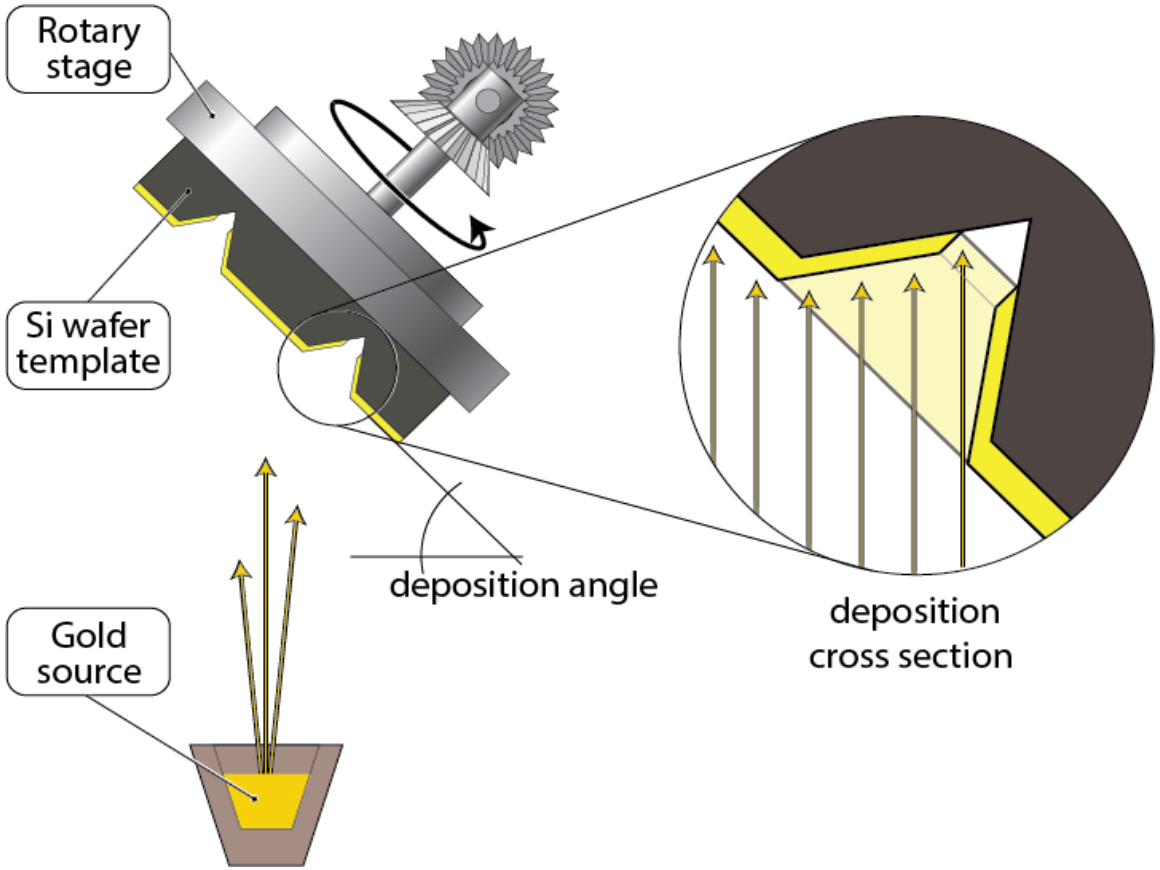
Inverted pyramids

Photolithography is used to fabricate large arrays of micropyramids

Chromium etch

Template

Gold is deposited at an angle to make tipless micropyramids



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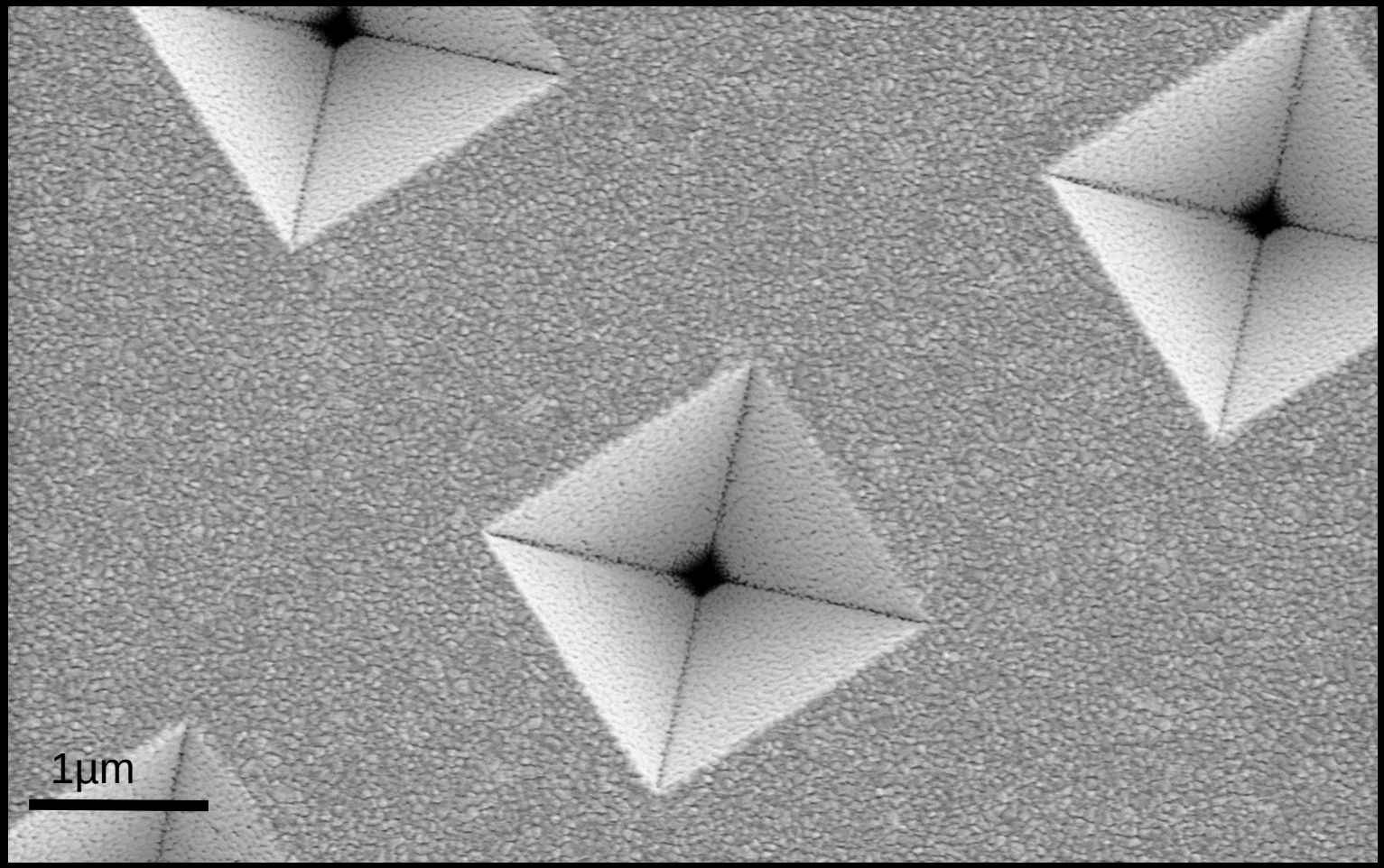
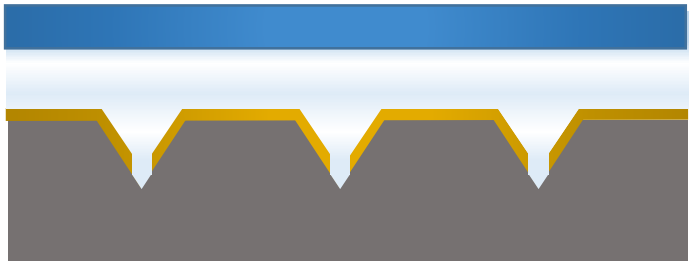


Template stripping produces final substrate

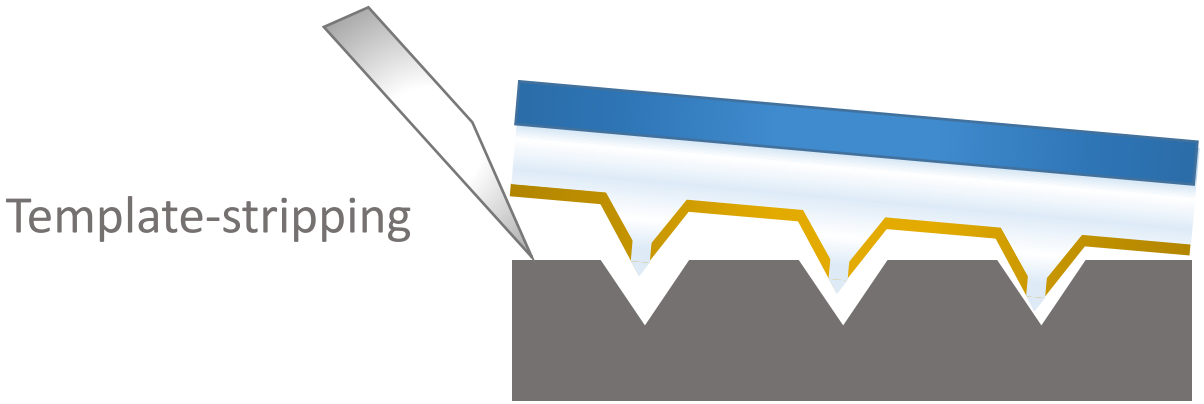
Template stripping produces final substrate

Glass coverslip

UV-cured glue

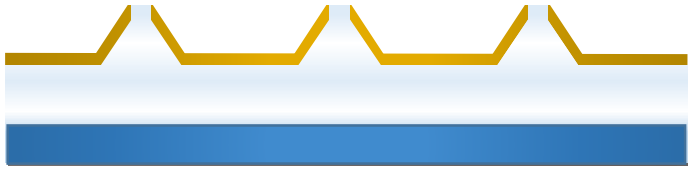


Template stripping produces final substrate

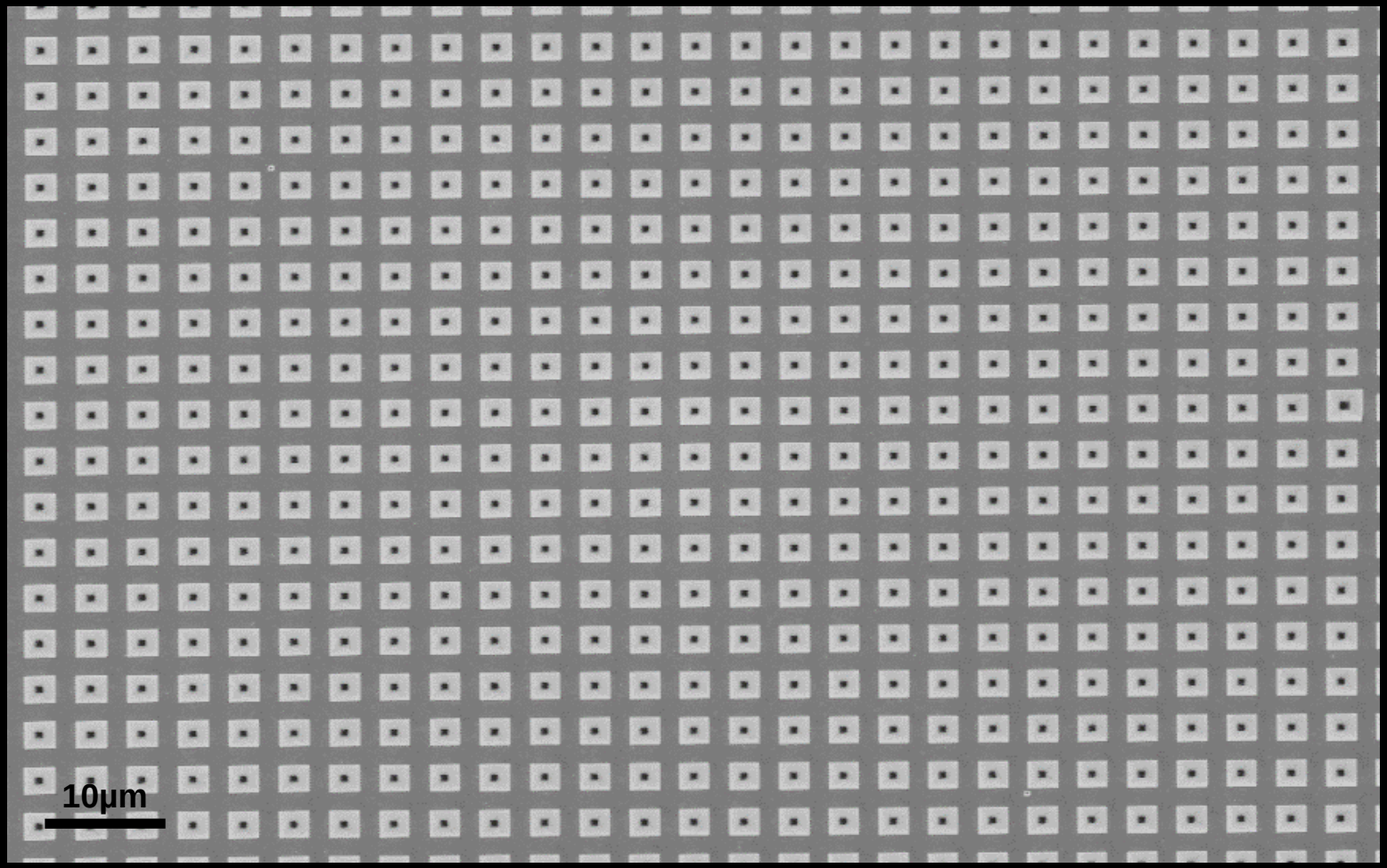


Template stripping produces final substrate

Tipless pyramids



Substrate has a large array of consistent pyramids



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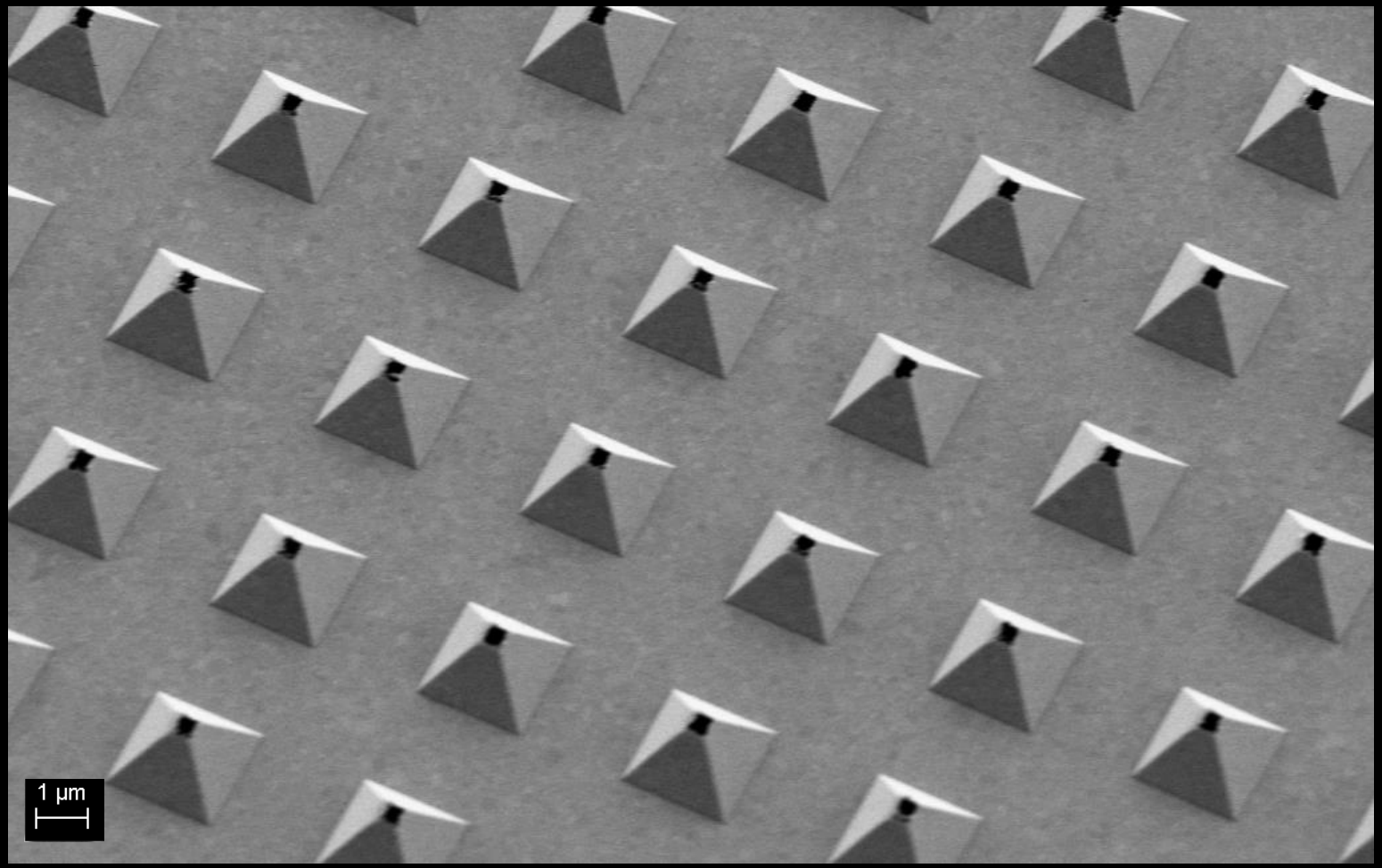
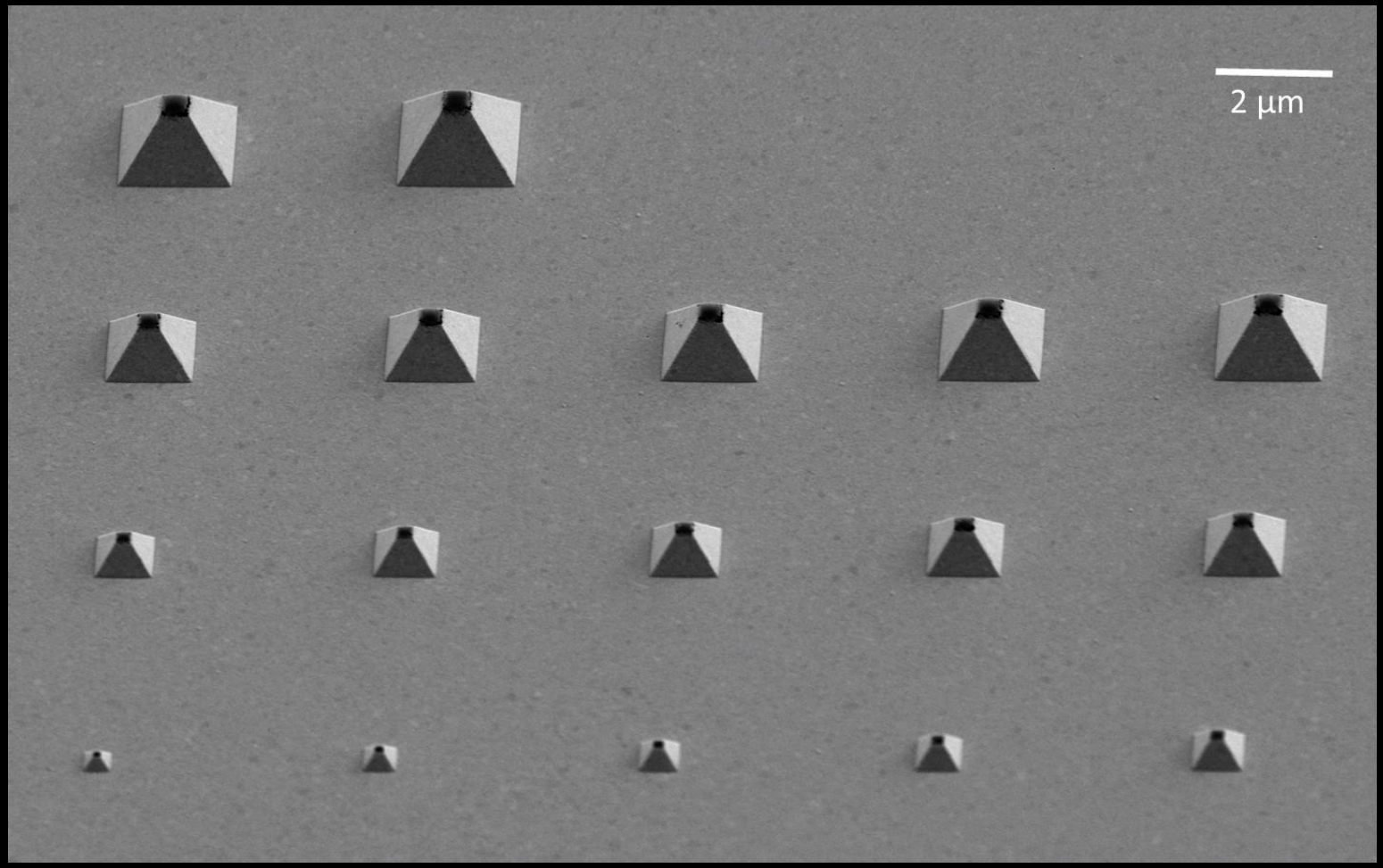
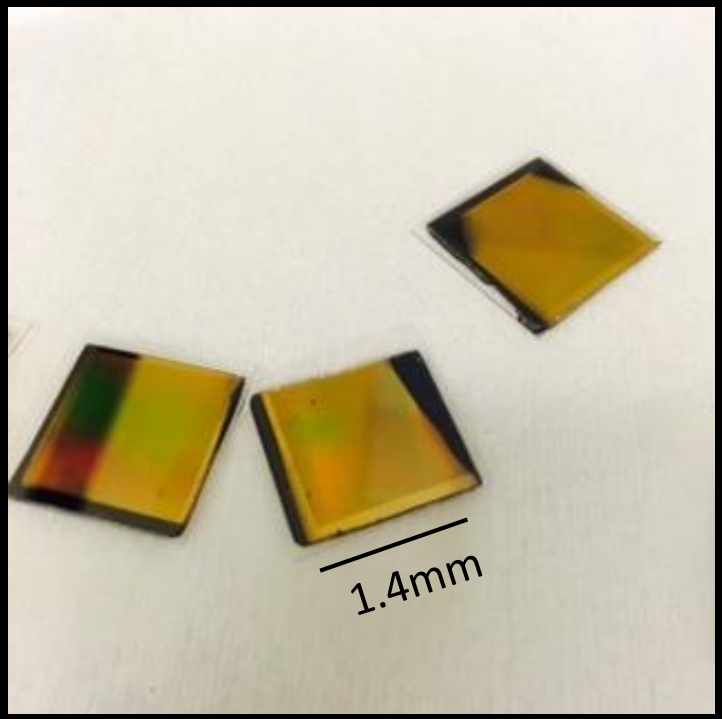
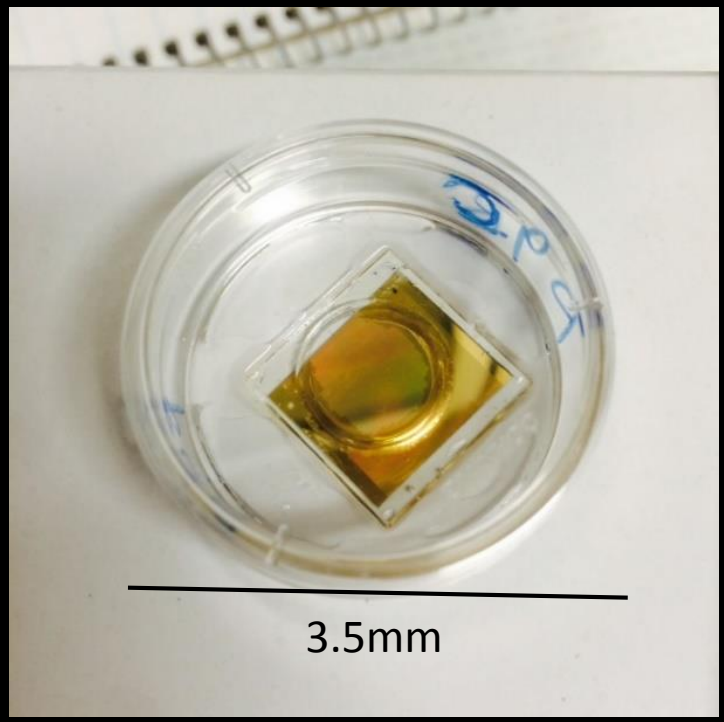


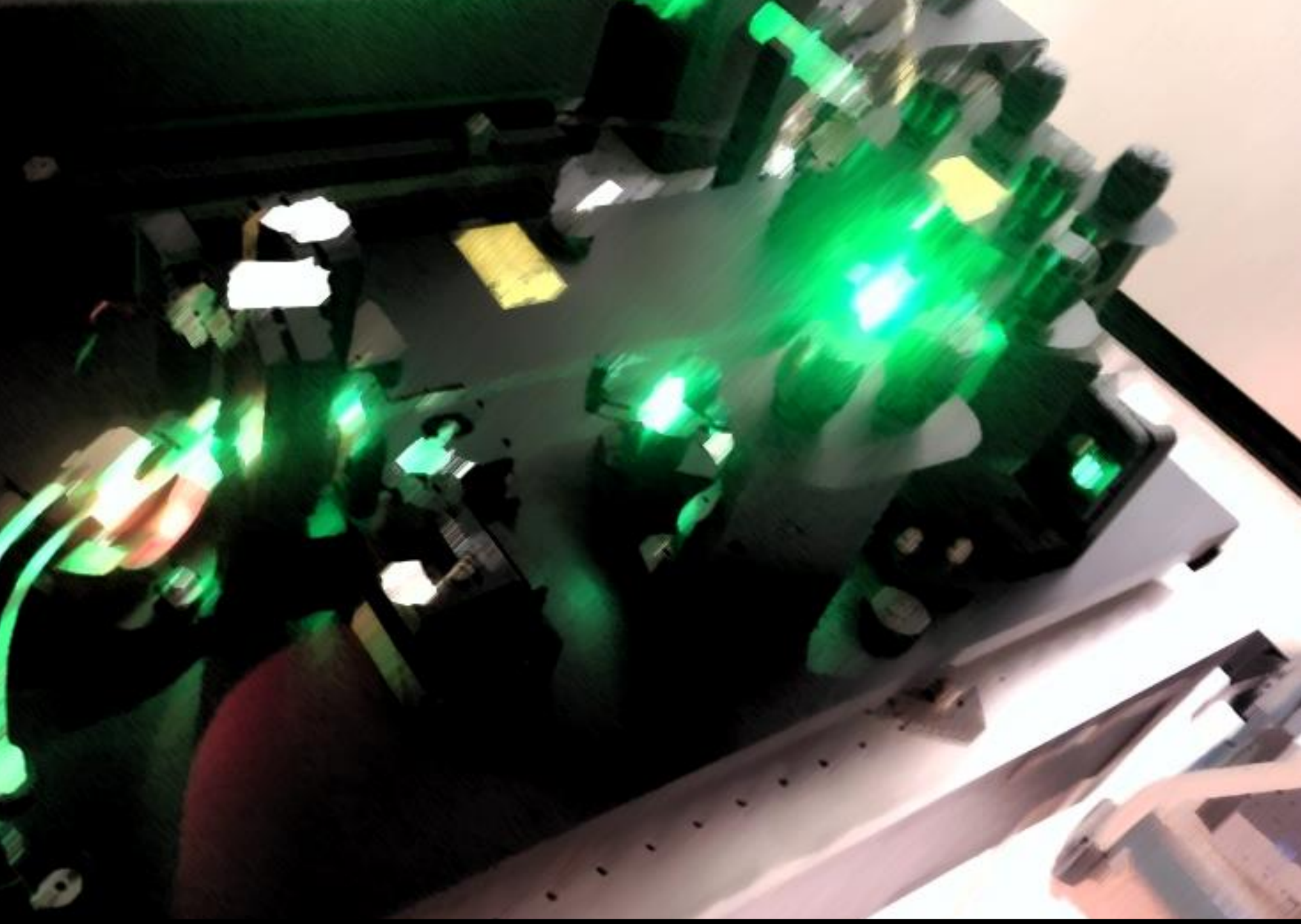
Pyramids can have different dimensions



Samples are ready for cell experiments

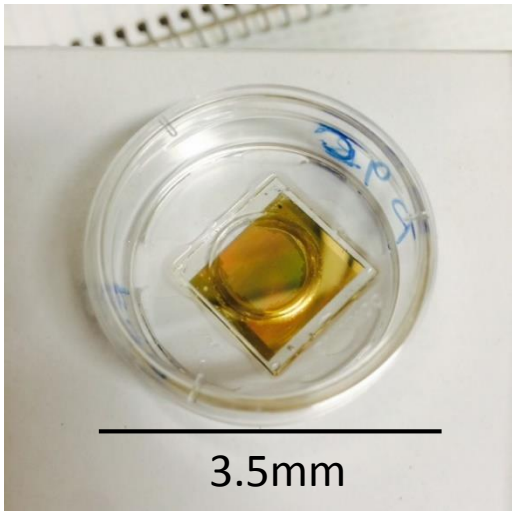




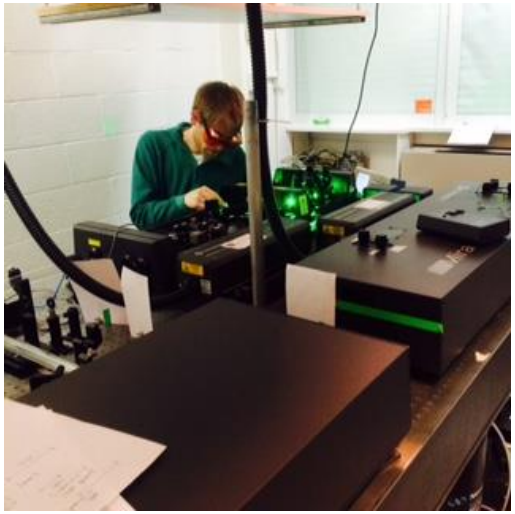


1 Introduction 2 Substrates 3 Experiments

Experiments have three components



Cell culture



Laser scanning

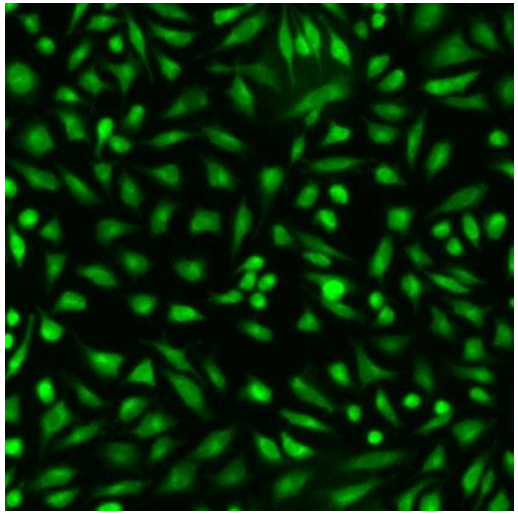
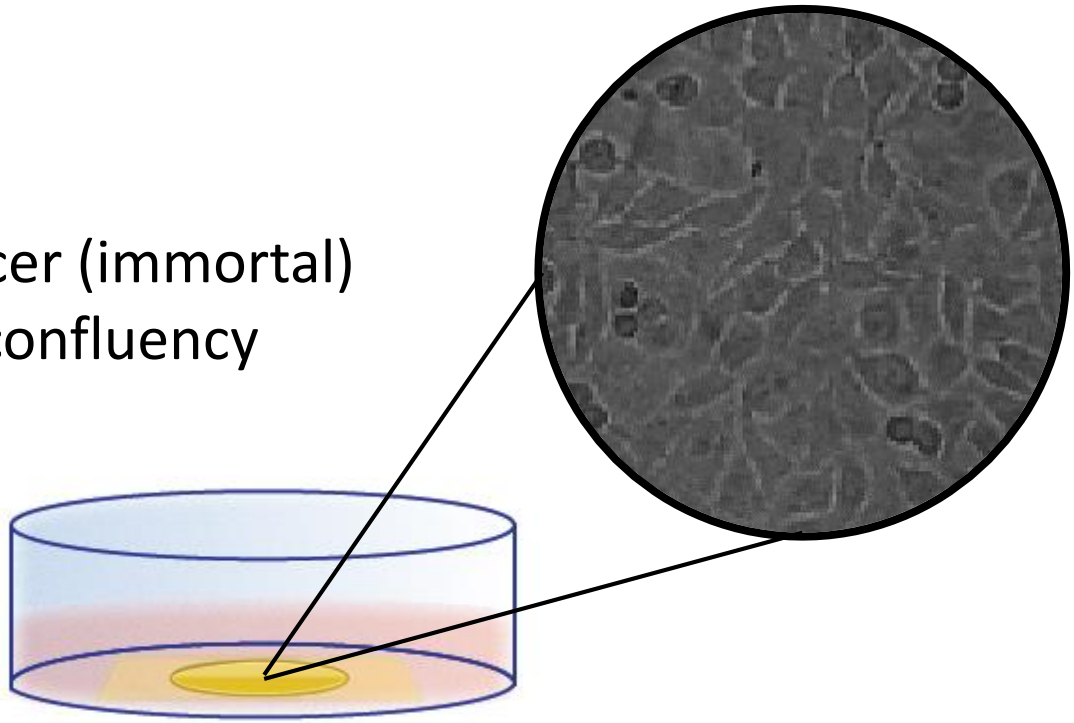


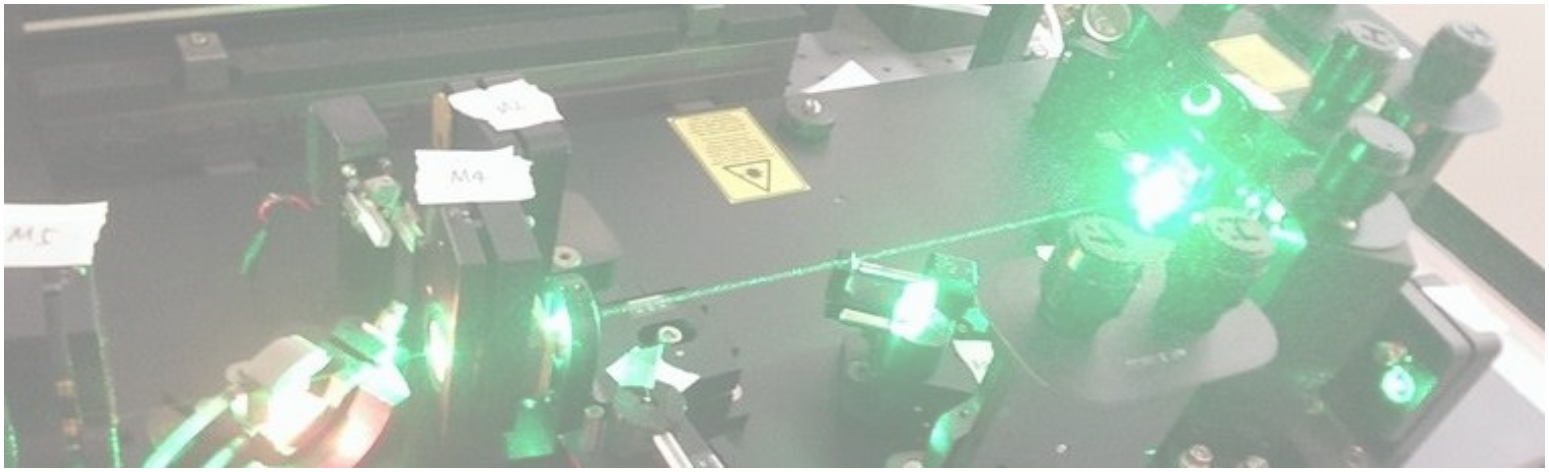
Image analysis

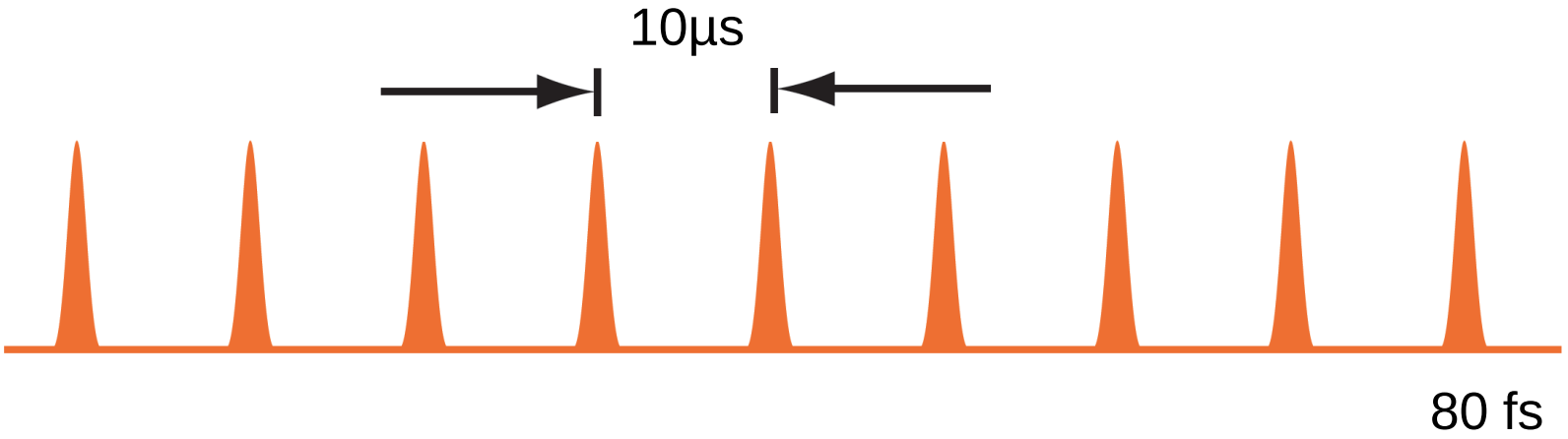
Cell culture on plasmonic substrates

cell line: HeLa
type: cervical cancer (immortal)
passaged at 80% confluency



Laser scanning with Ti: sapphire laser





Laser scanning with Ti: sapphire laser

pulse duration: 80 fs

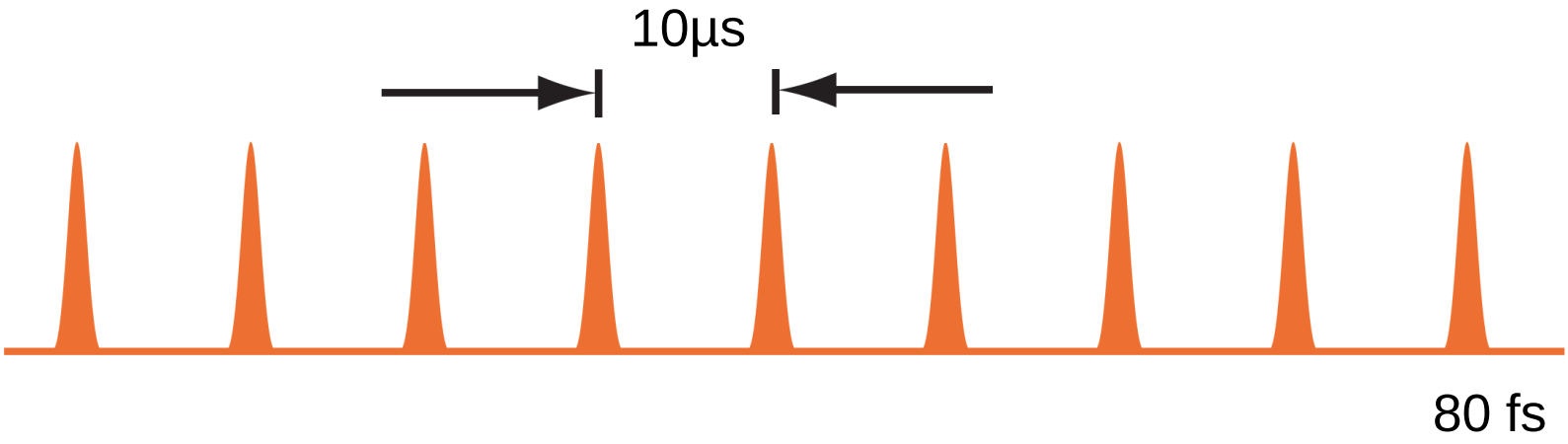
repetition rate: 100 kHz

average power: 300 mW

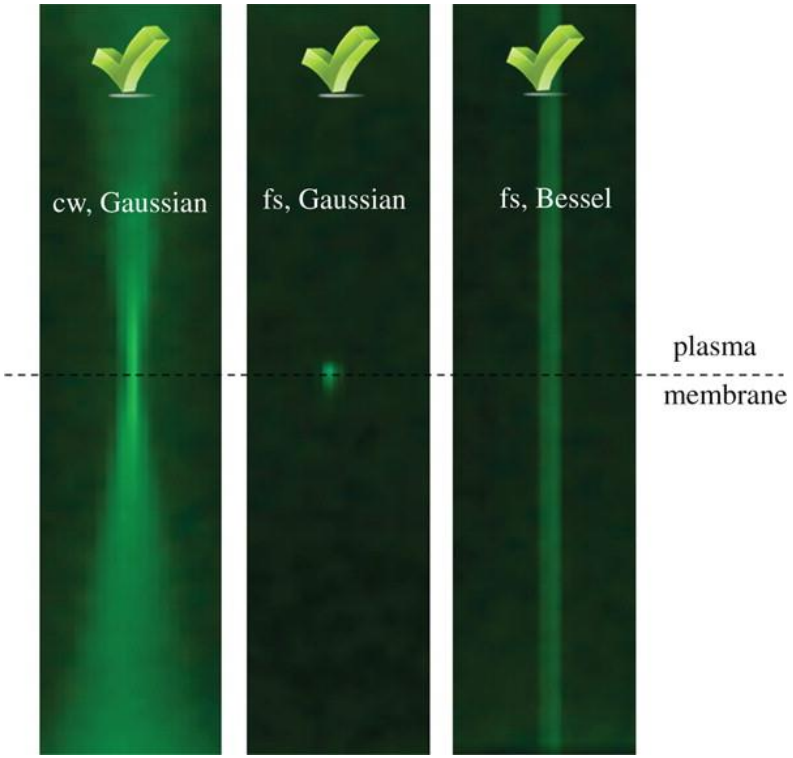
peak power: 10^6 W

energy per pulse: 3µJ

wavelength: 800 nm



A Gaussian beam allows us to scan small areas



Single cell optical transfection- D. Stevenson et al., J. R. Soc. Interface:2010

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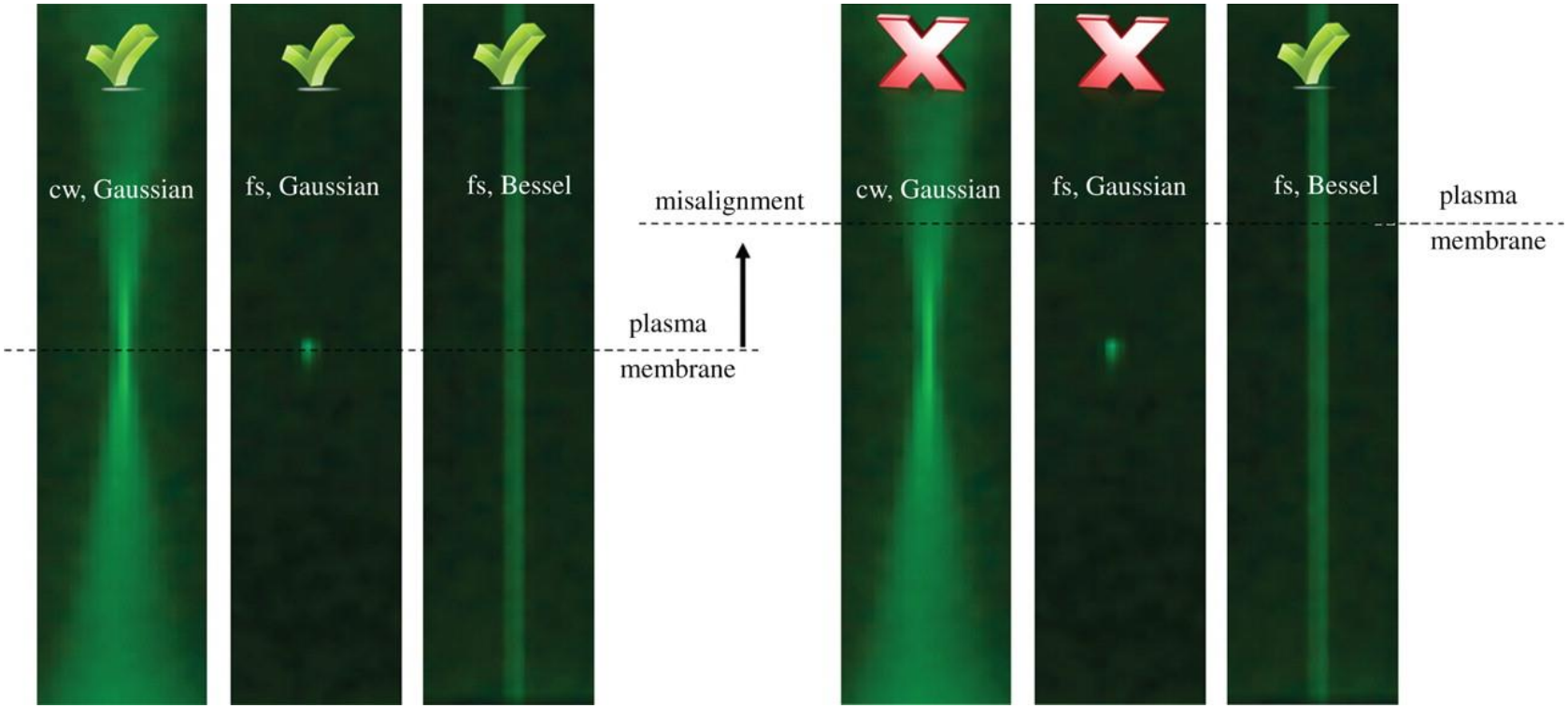
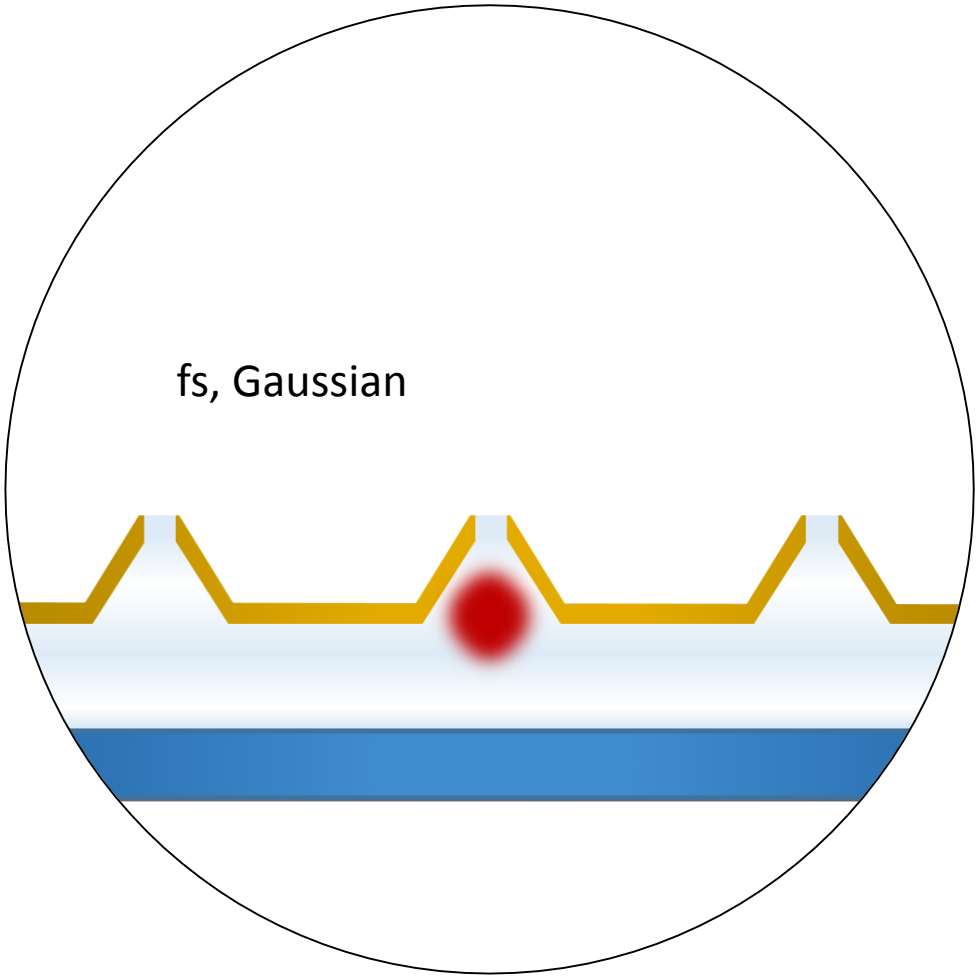


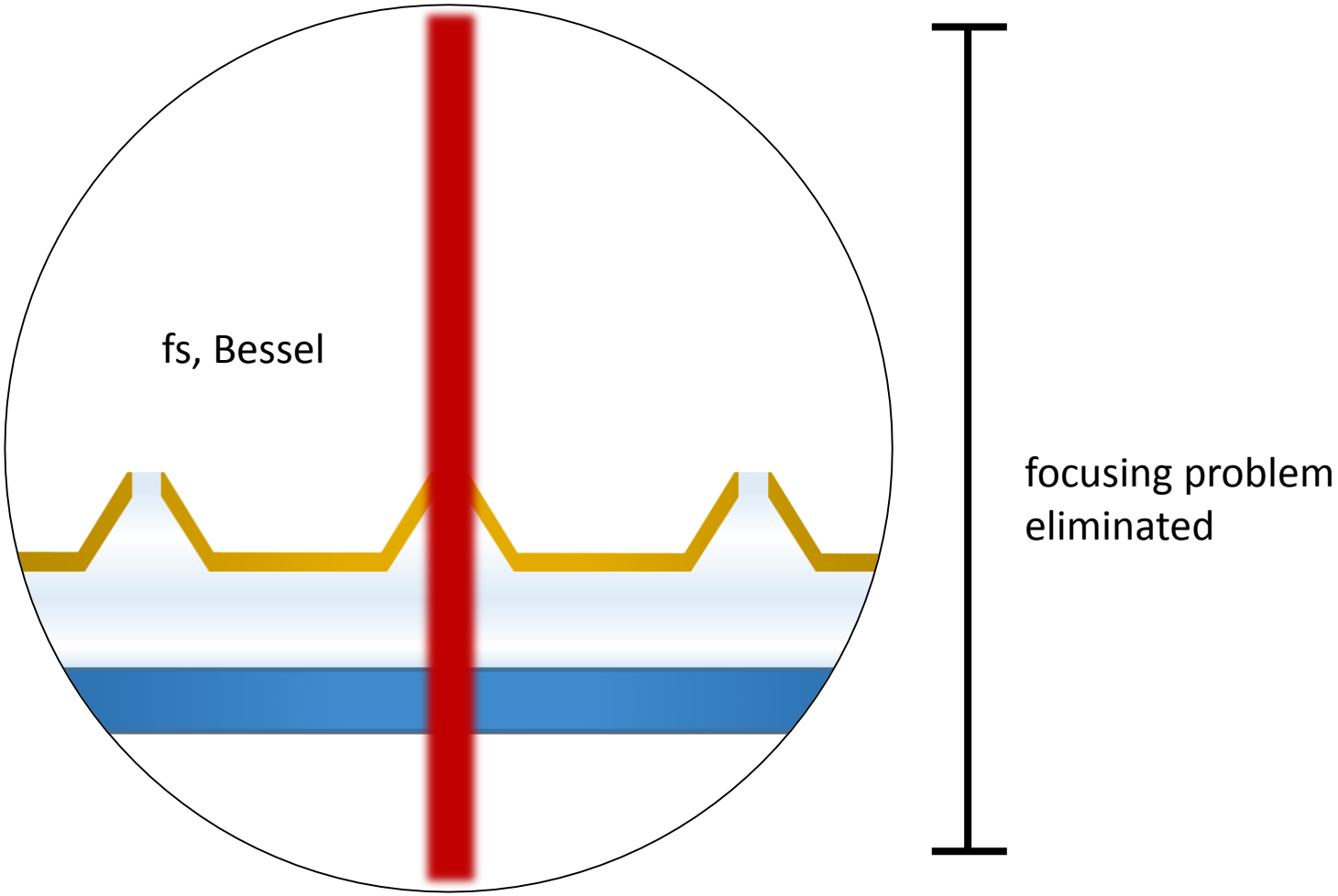
Single cell optical transfection- D. Stevenson et al., J. R. Soc. Interface:2010

A Gaussian beam allows us to scan small areas

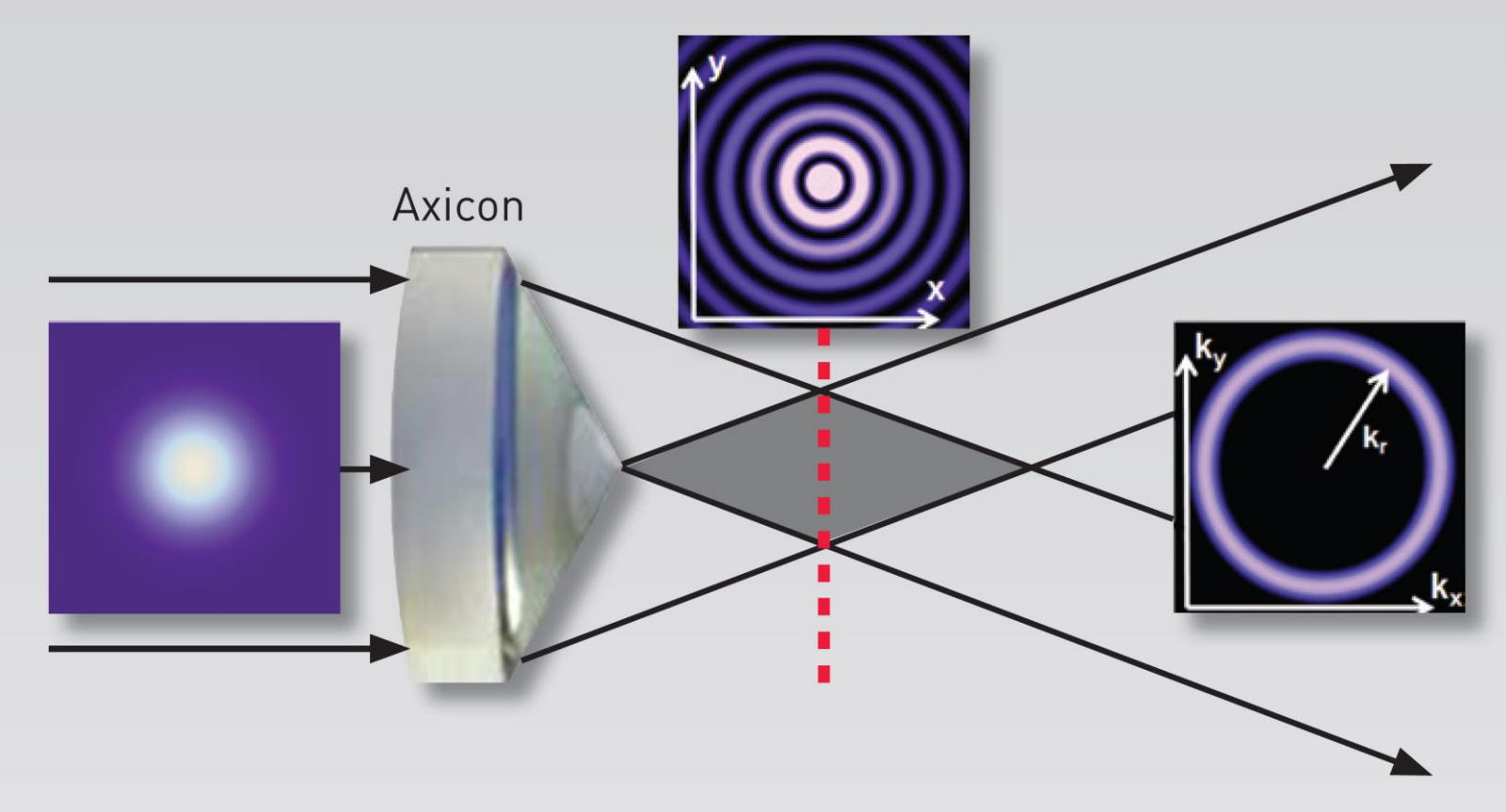


Misalignment of even a few microns inhibits plasmonic enhancement

A non-diffracting light beam allows us to scan large areas

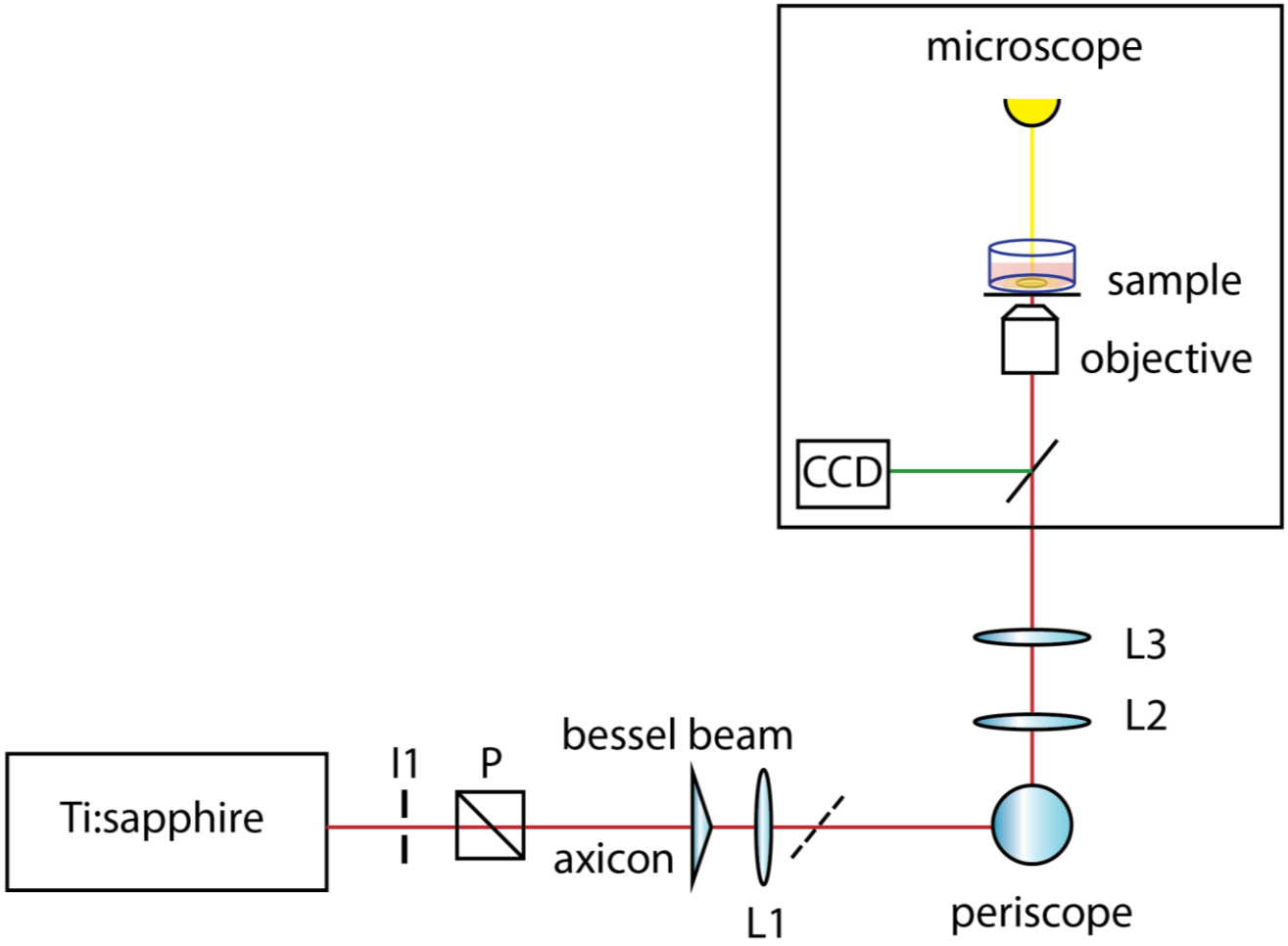


A non-diffracting light beam allows us to scan large areas

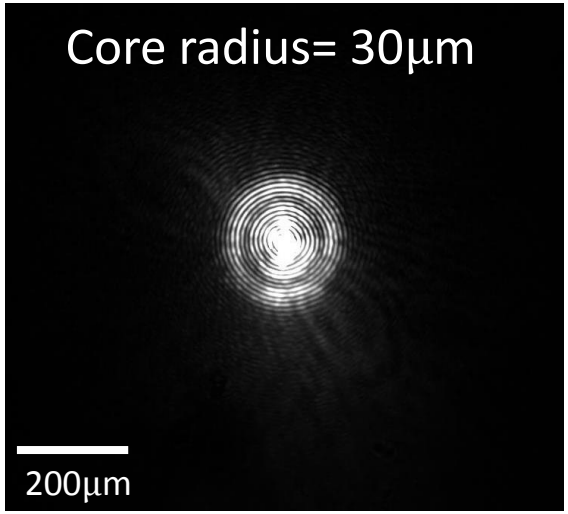


Dudley, Angela, et al. "Unraveling Bessel beams." *Optics and Photonics News*24.6 (2013): 22-29.

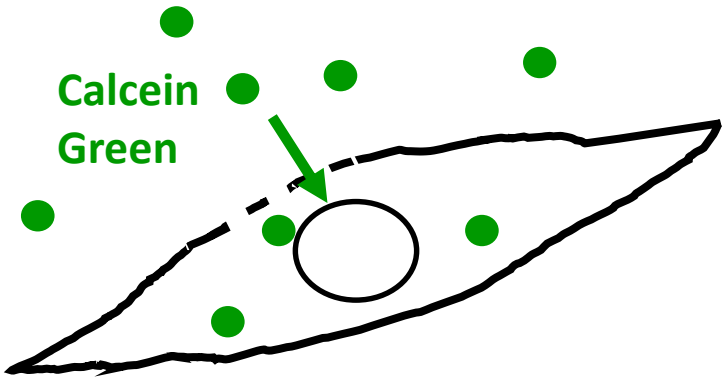
A non-diffracting light beam allows us to scan large areas



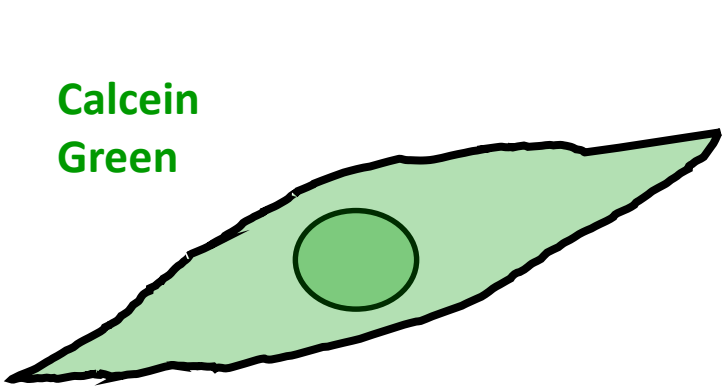
A non-diffracting light beam allows us to scan large areas



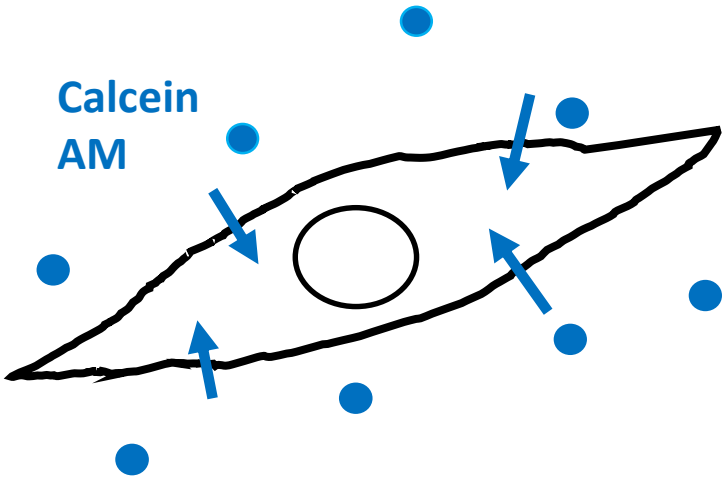
Quantification of cell poration and viability using dye molecules



Quantification of cell poration and viability using dye molecules

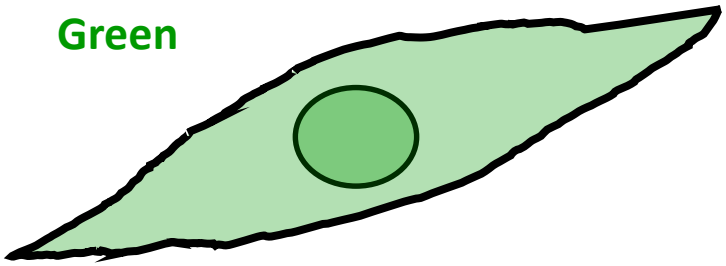


porated cell



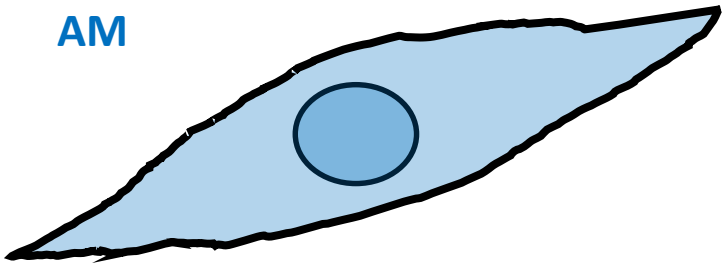
Quantification of cell poration and viability using dye molecules

Calcein Green



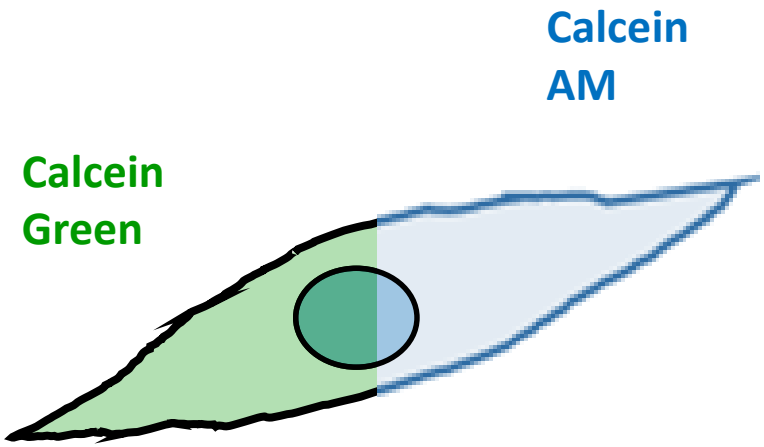
porated cell

Calcein AM



Viable cell

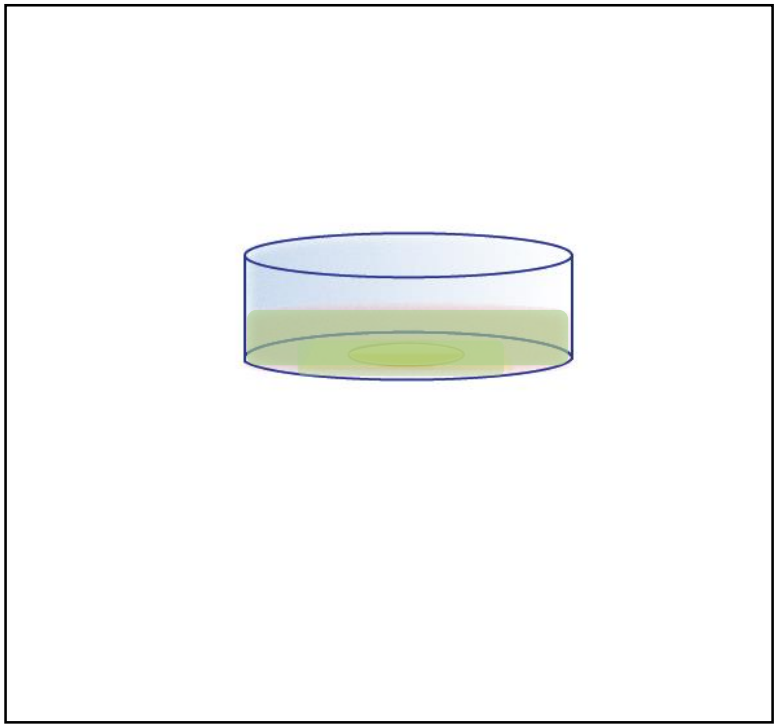
Quantification of cell poration and viability using dye molecules



porated + viable cell

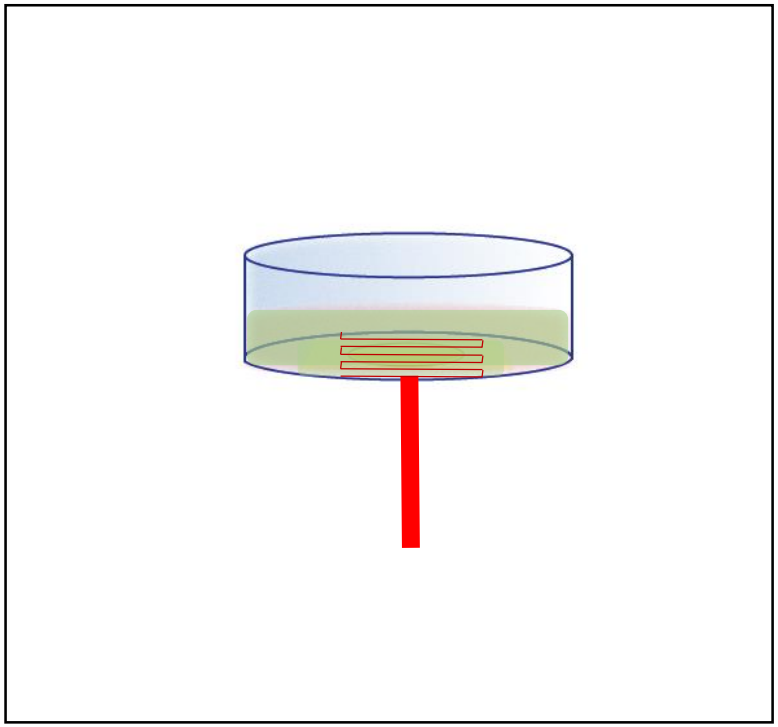
Experimental procedure

1) Add calcein green
2) Laser treatment
3) Washing step
4) Add calcein AM
5) Imaging



Experimental procedure

1) Add calcein green
2) Laser treatment
3) Washing step
4) Add calcein AM
5) Imaging



Experimental procedure

1) Add calcein green
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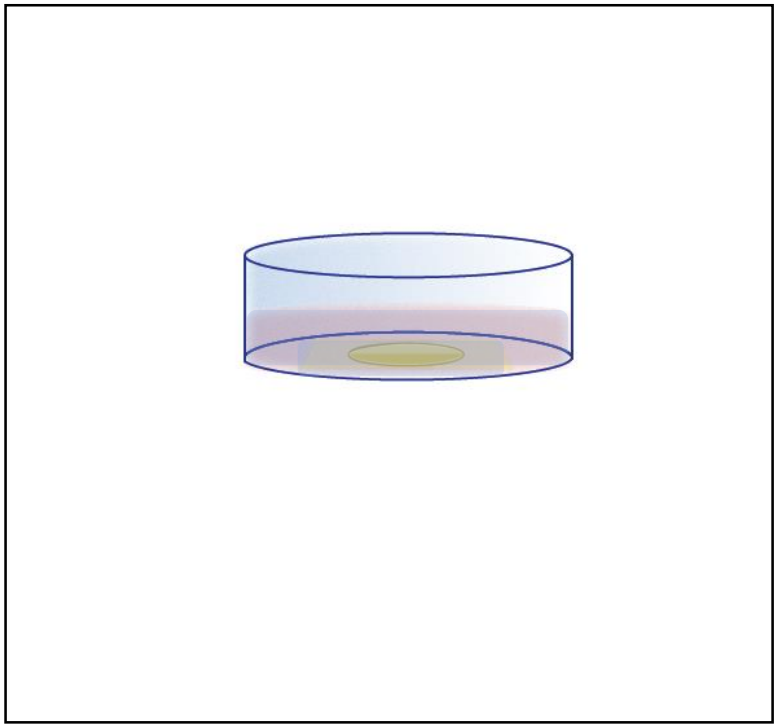
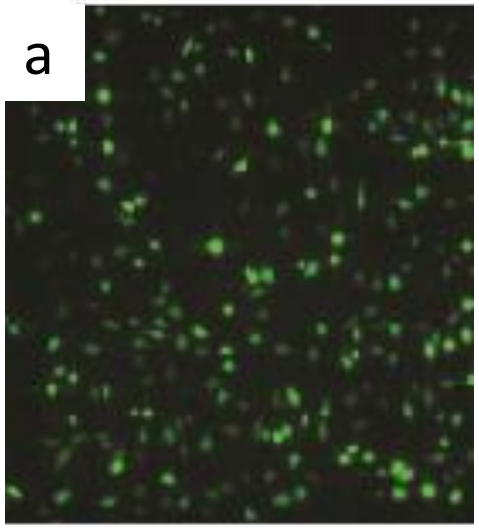
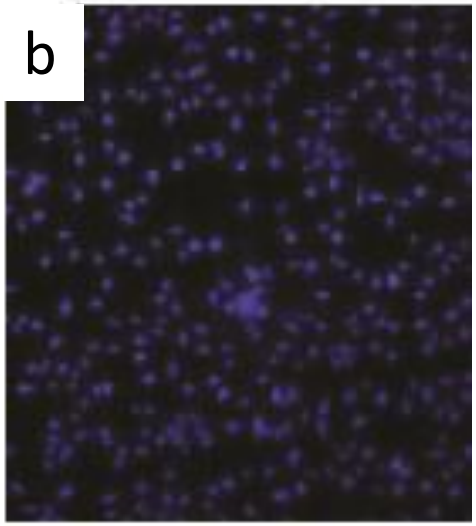
5) Imaging



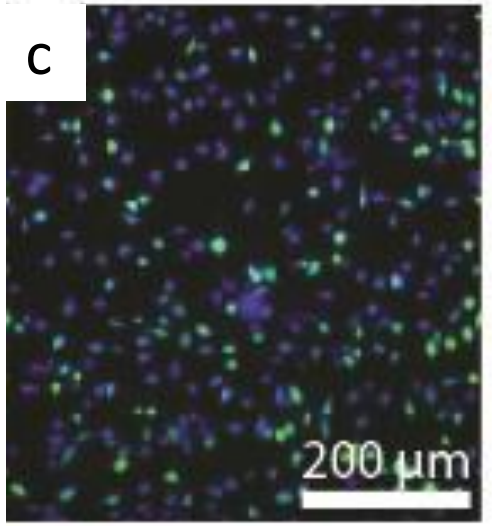
Fluorescence microscopy to image poration and viability



Porated

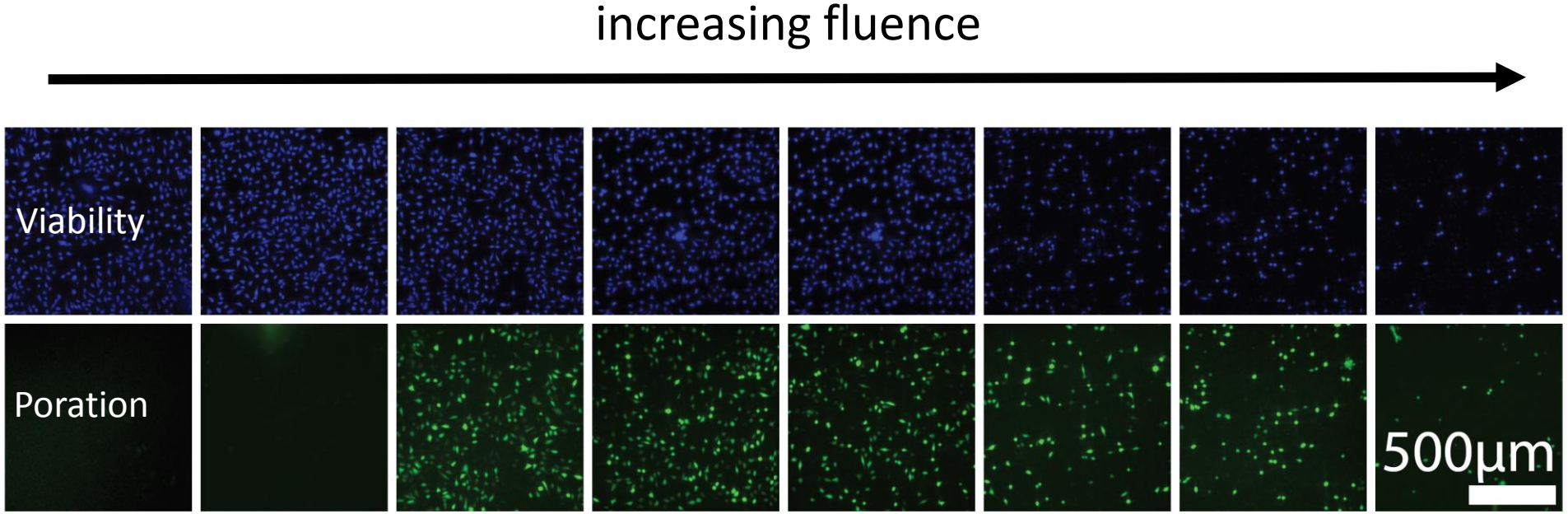


Viable

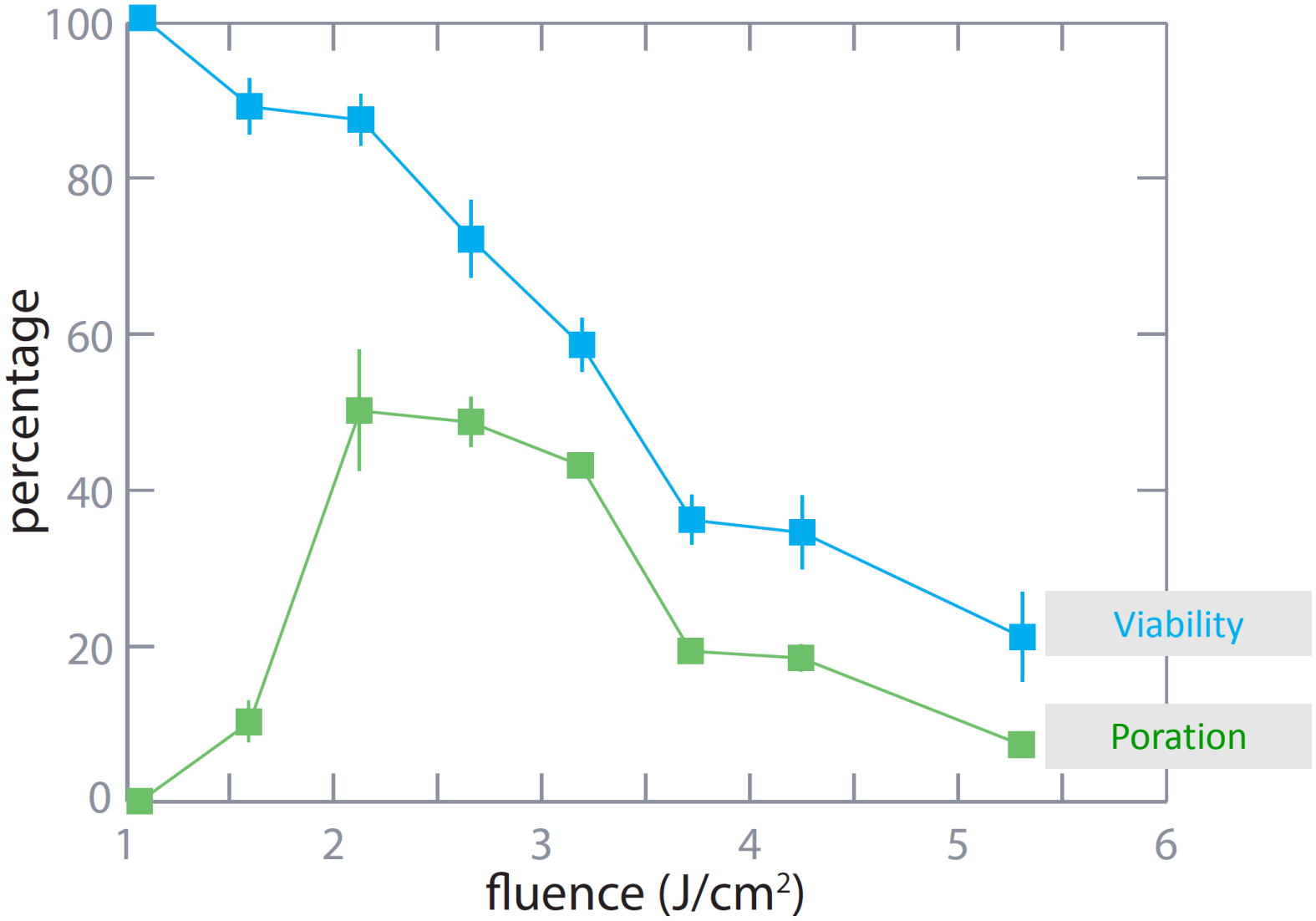


Porated + Viable

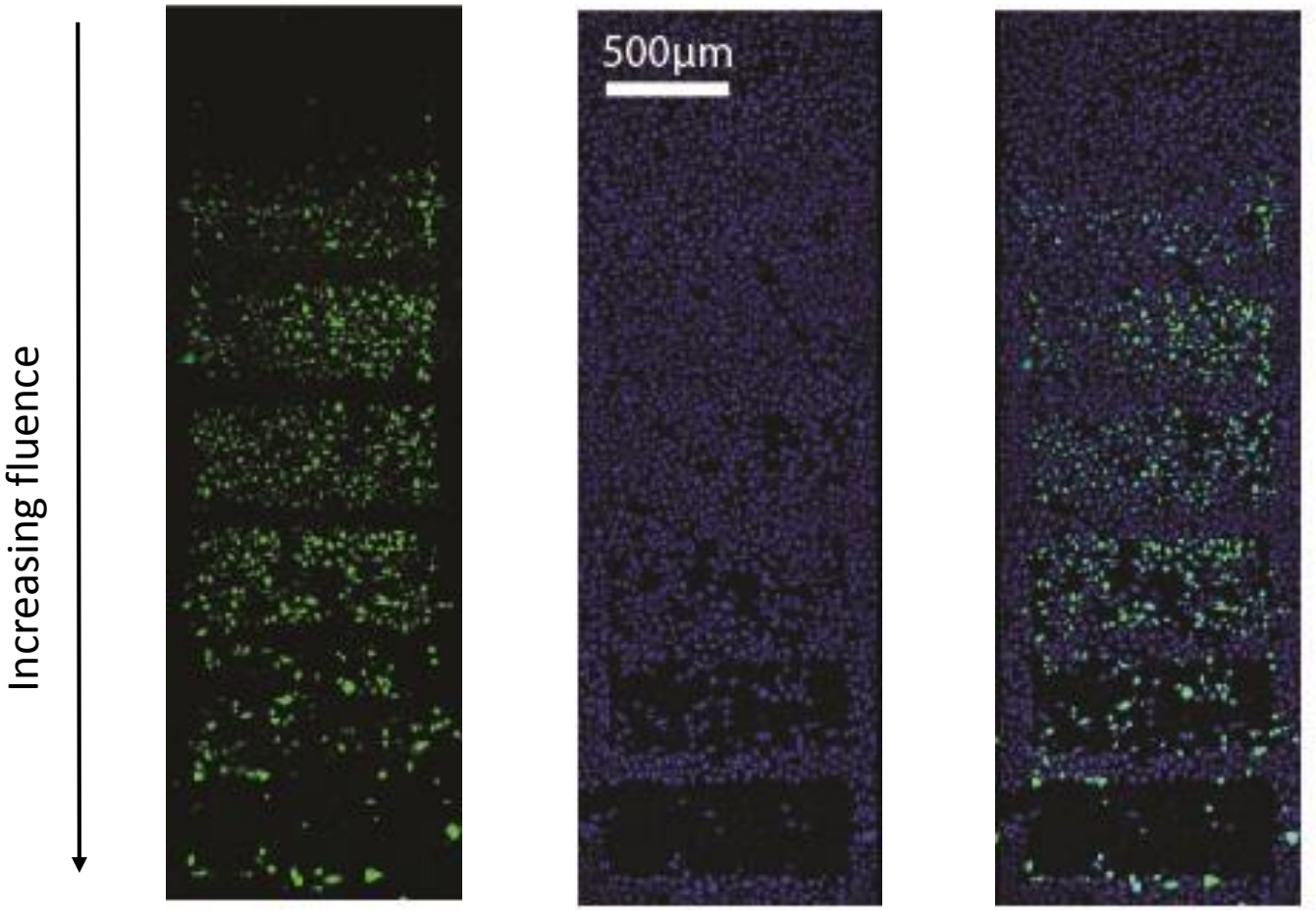
Change in fluence affects poration and viability



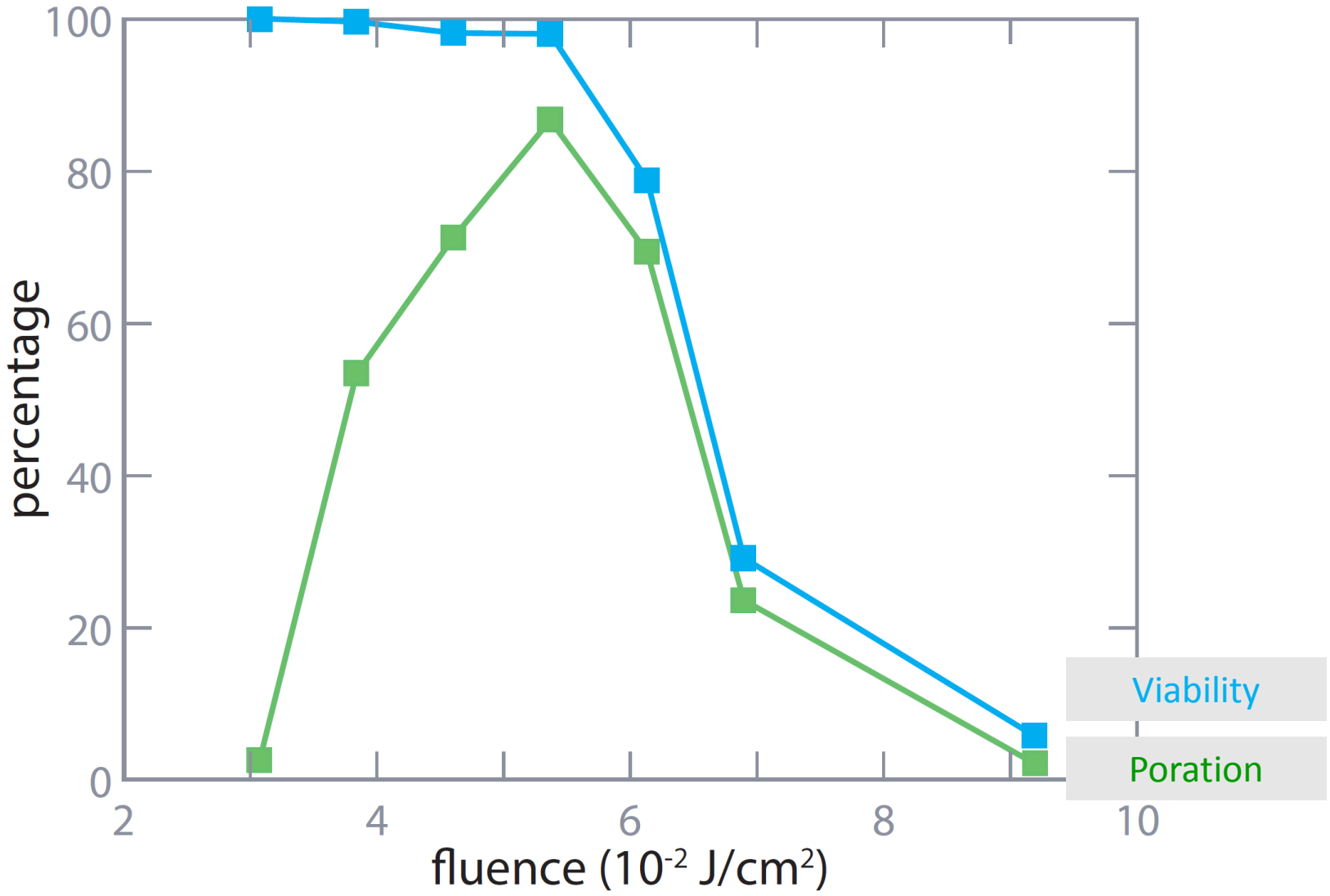
50% of HeLa cells porated with a 40x objective



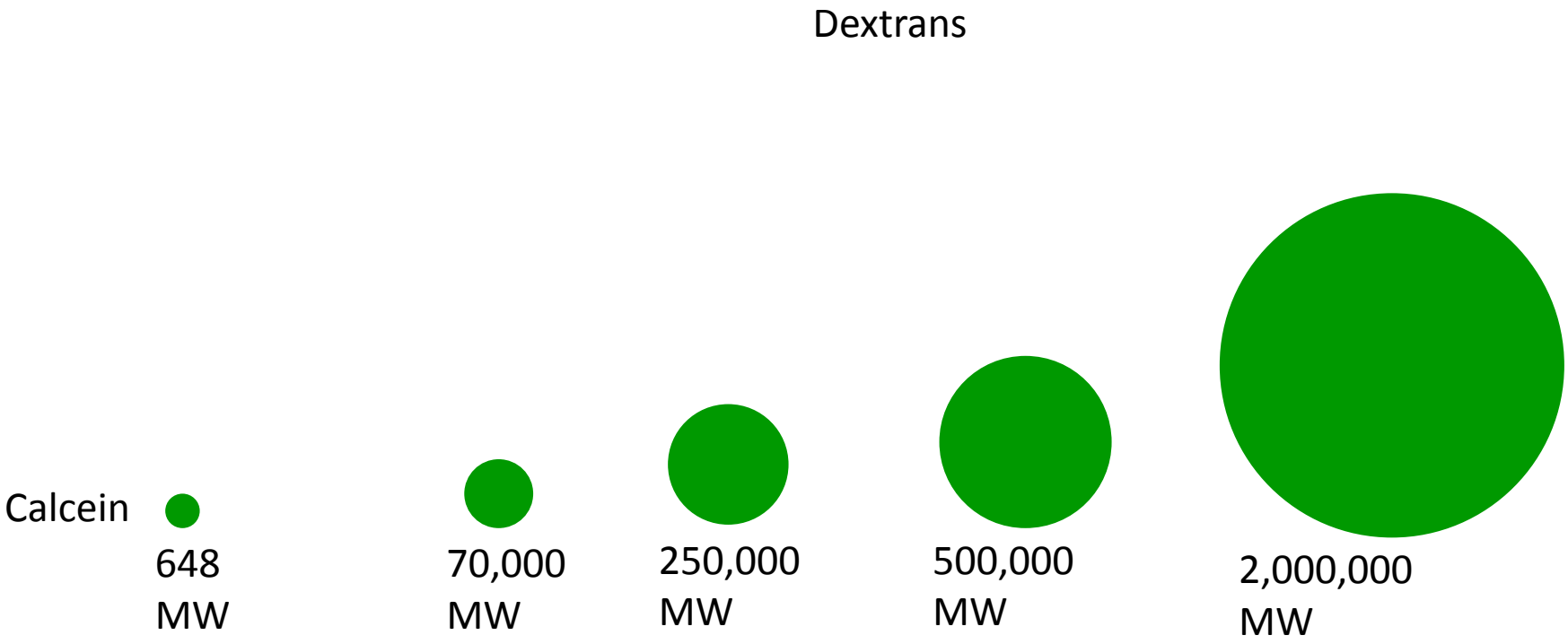
Optimizing with 4x objective



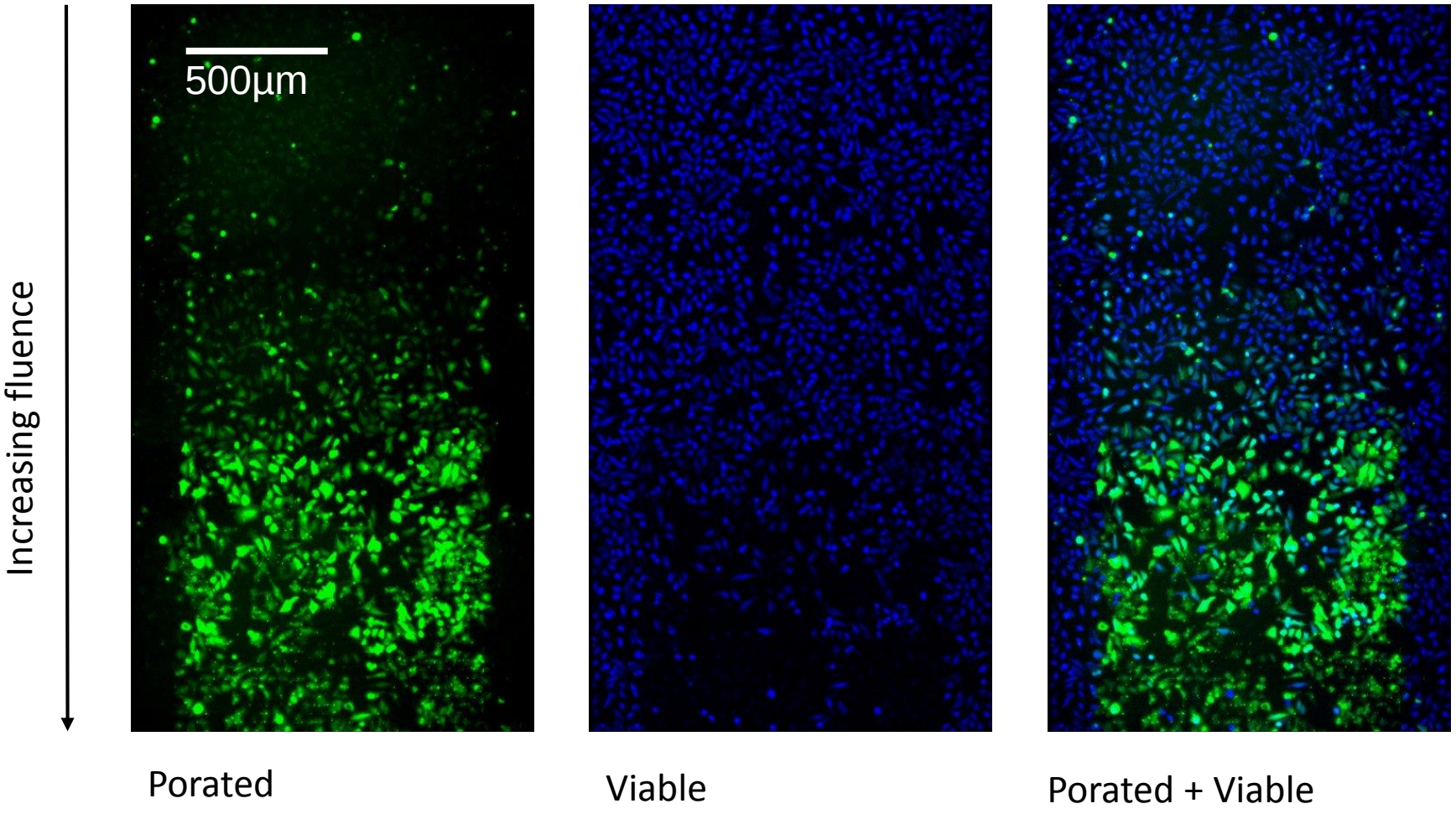
85% of HeLa cells porated with a 4x objective



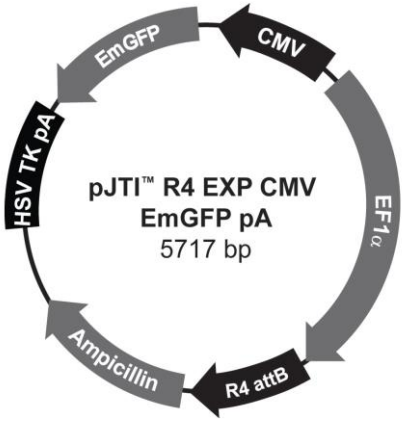
Experiments in progress:
different Dextrans to determine pore size



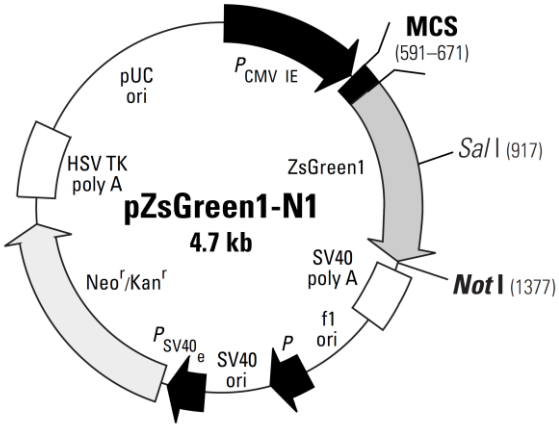
Introducing larger dyes: Dextran 70,000 MW



Experiments in progress: transfection with DNA plasmids

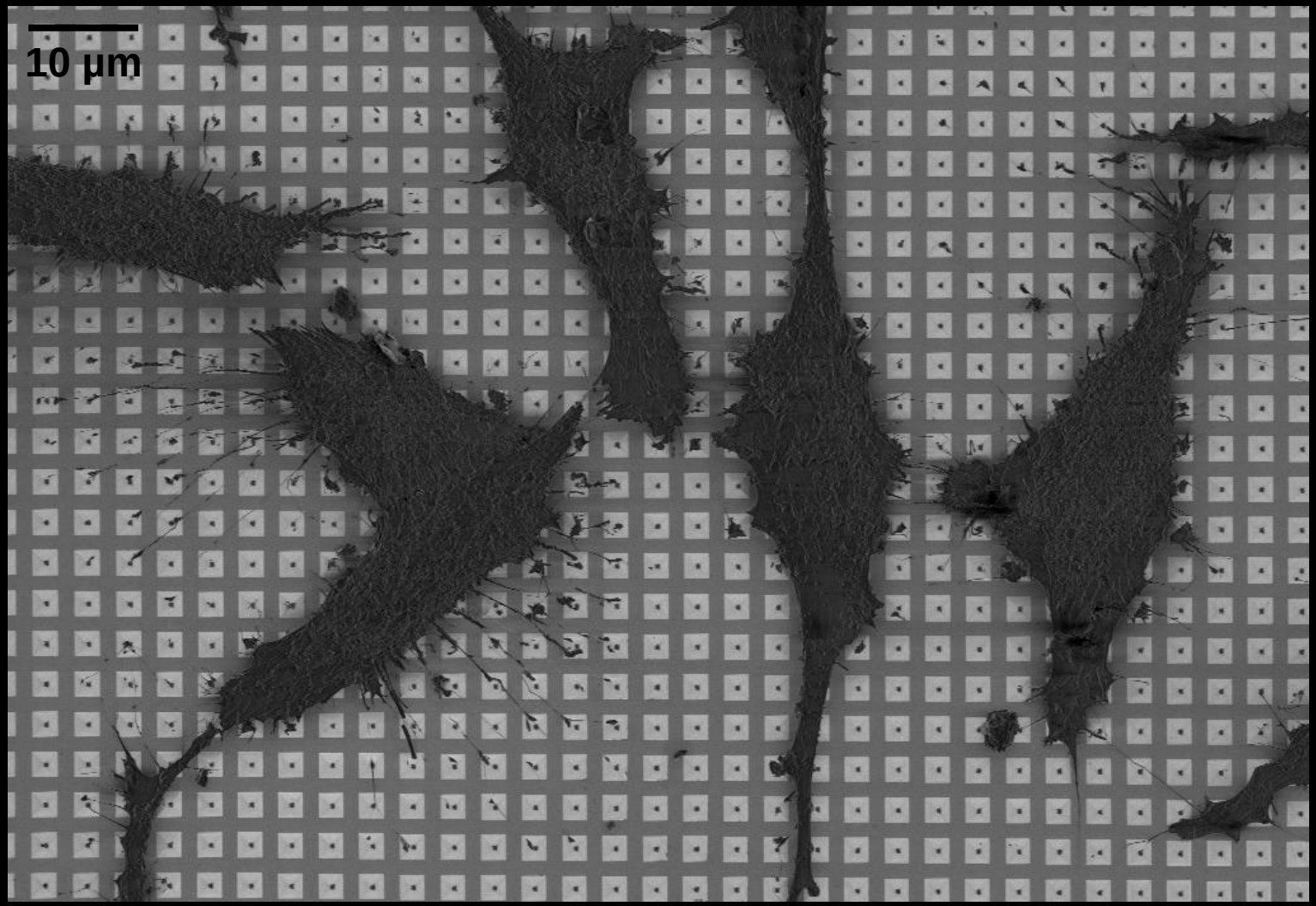


Transient
GFP expression

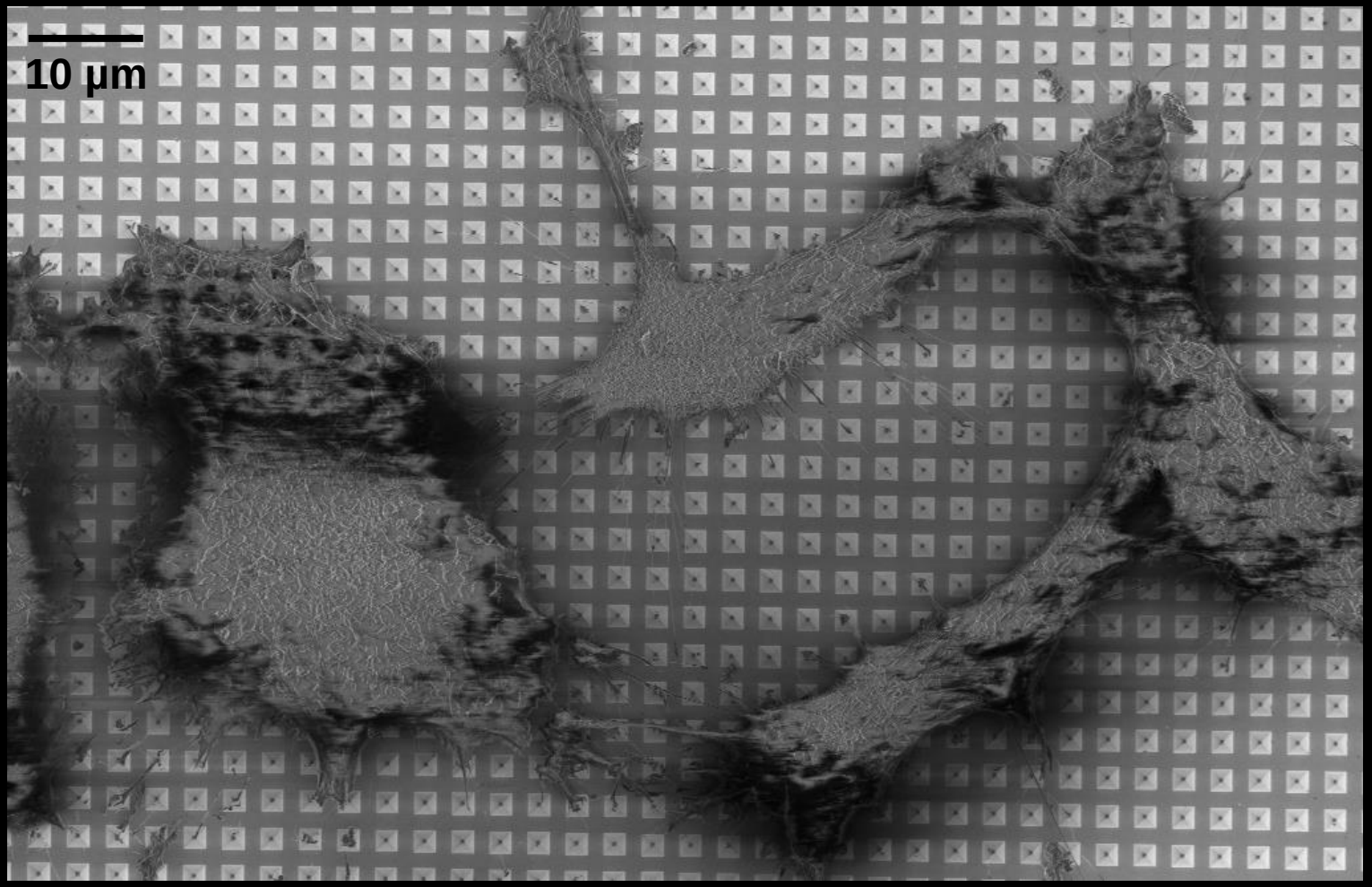


Permanent
GFP expression

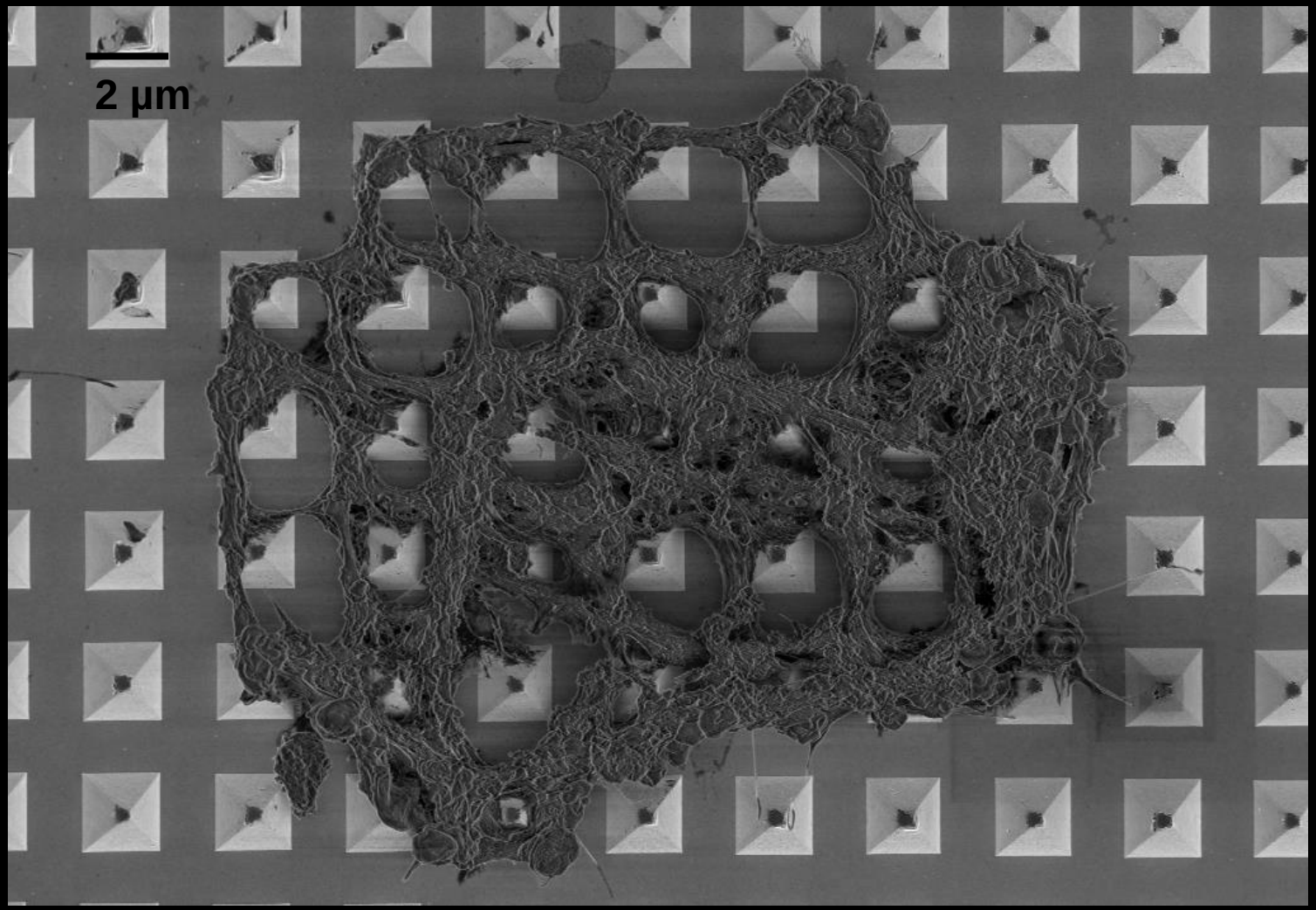
Membrane-substrate interactions determine poration success



Cells uptake dye molecules through pores in membrane



Cells killed by too many large pores

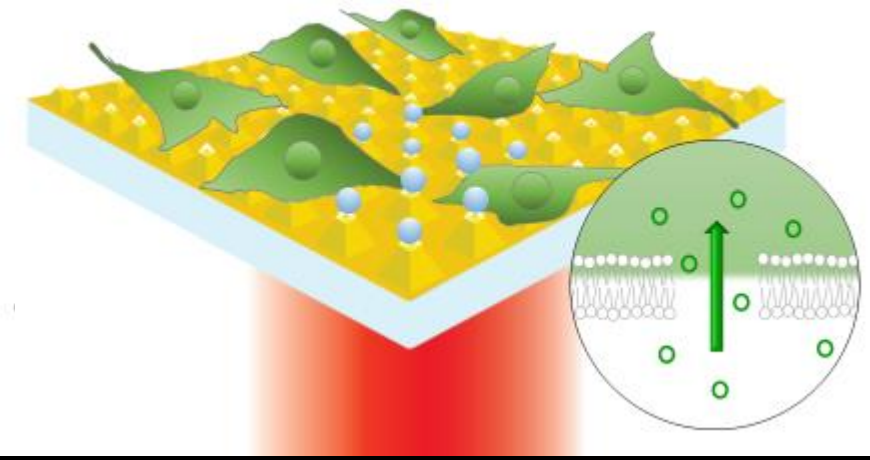


Outlook

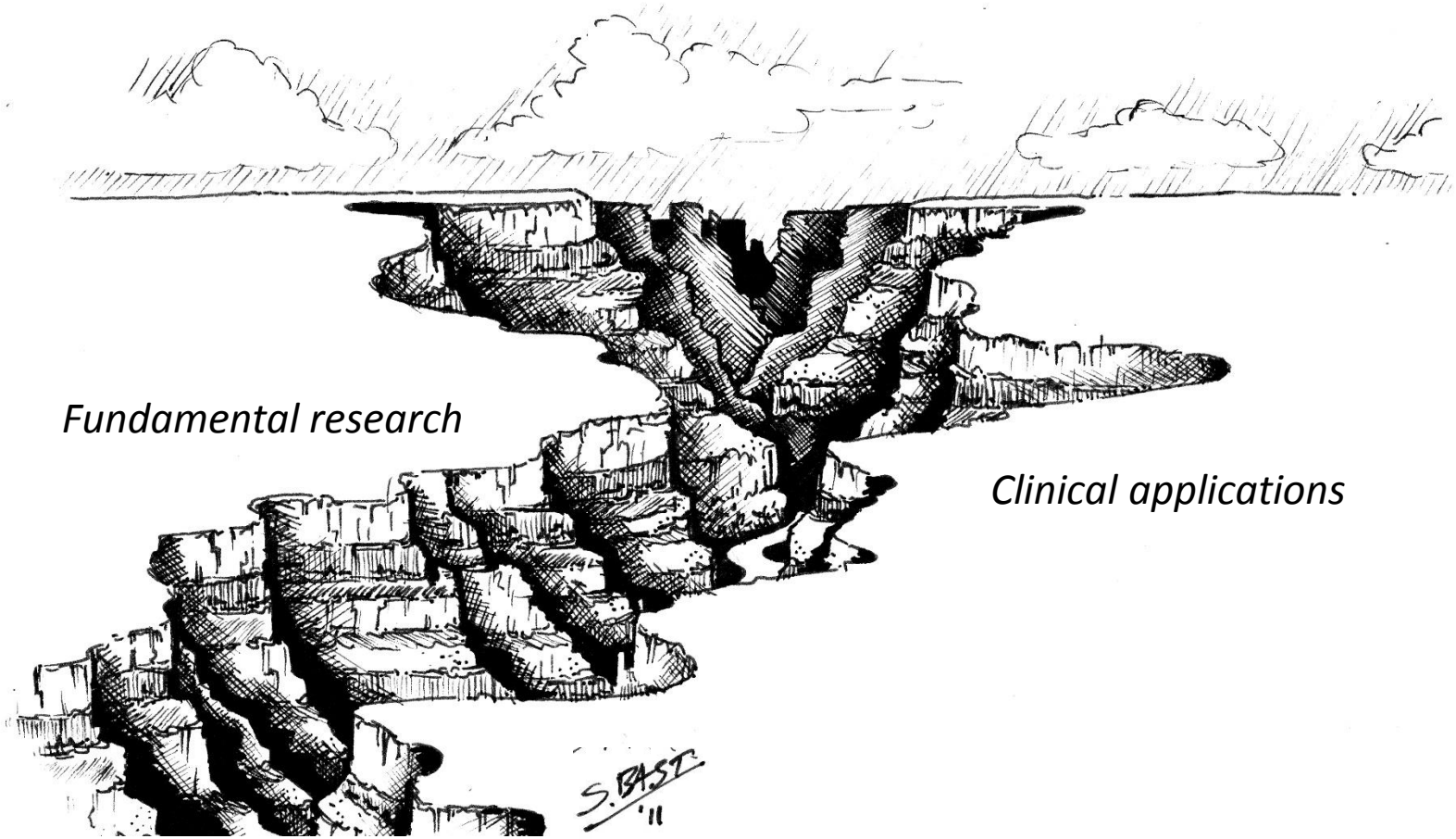
New approach towards cell transfection

Poration → *Transfection*

High throughput
High efficiency
High viability



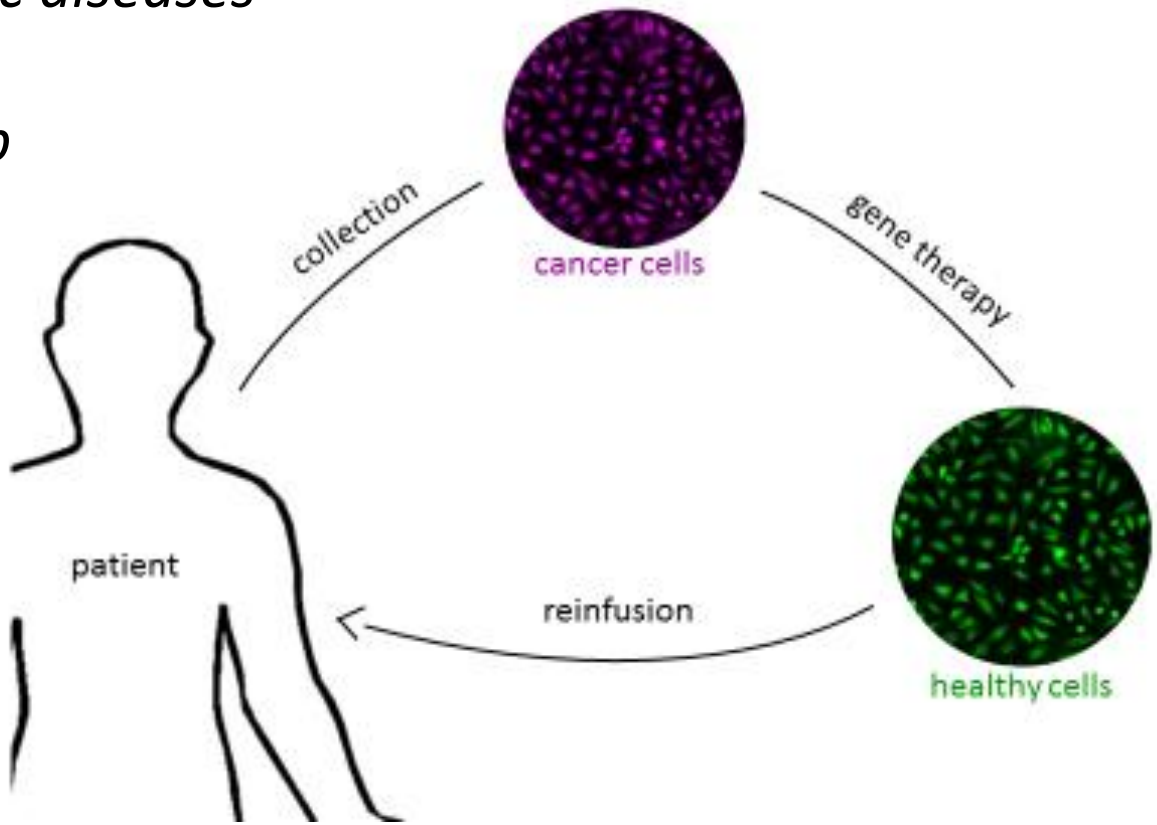
Making the leap from fundamental research to clinical applications



Going towards disease-focused applications

Target specific diseases

Lab-on-a-chip



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Daryl Vulis

Marinna Madrid

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Prof. Michel Munier, Polytechnique Montreal
Dr. Alain Viel, Harvard biolabs
Prof. Chris Schaffer, Cornell University
Weilu Shen, RPI
Lauren Milling, UIUC

Special thanks to the Mazur group

Alex Raun, Harvard University (add pic)!!!

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Daryl Vulis

Marinna Madrid

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Howard Hughes Medical Institute

American Association of University Women

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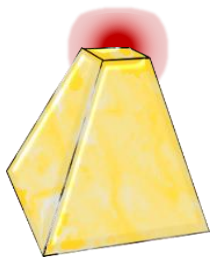
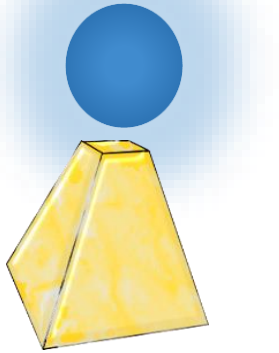
Extra slides

Simulations to understand the temperature evolution on structures



Plasmonic enhancement

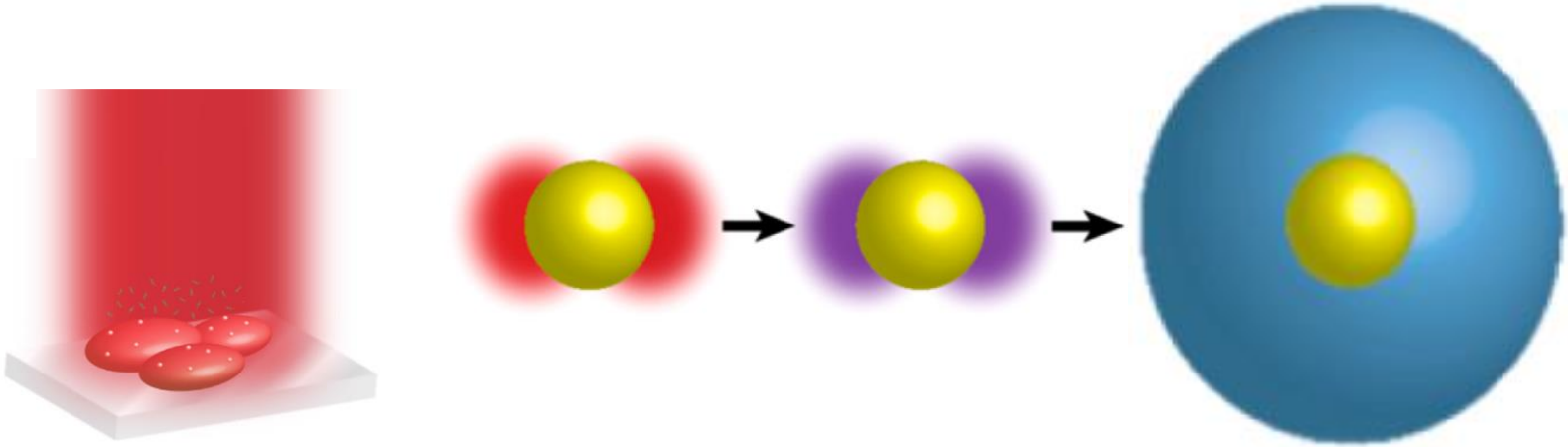
Plasma



Nanobubbles

Relate to poration and transfection

Simulations to understand the temperature evolution on structures



From Thermo- to Plasma-Mediated Ultrafast Laser-Induced Plasmonic Nanobubbles, R. Lachaine, Etienne Boulais, and Michel Munier

Simulations to understand the temperature evolution on structures

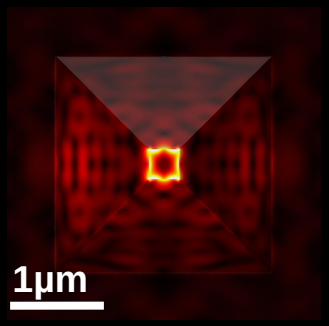
Electric field
Temperature Model
Plasma Formation
Hydrodynamic Model

High Throughput Poration of Mammalian Cells using Femtosecond Laser-activated Plasmonic Substrates

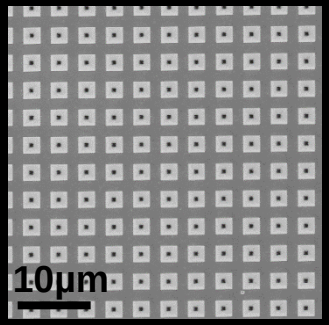
Nabiha Saklayen

Department of Physics at Harvard University

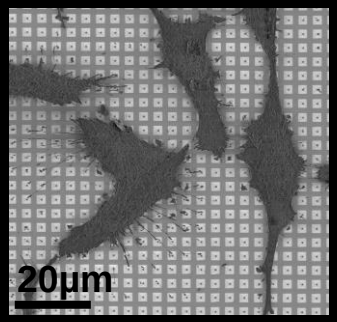
Simulations



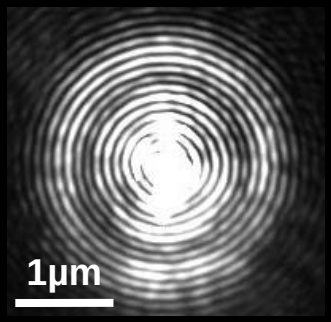
Nanofabrication



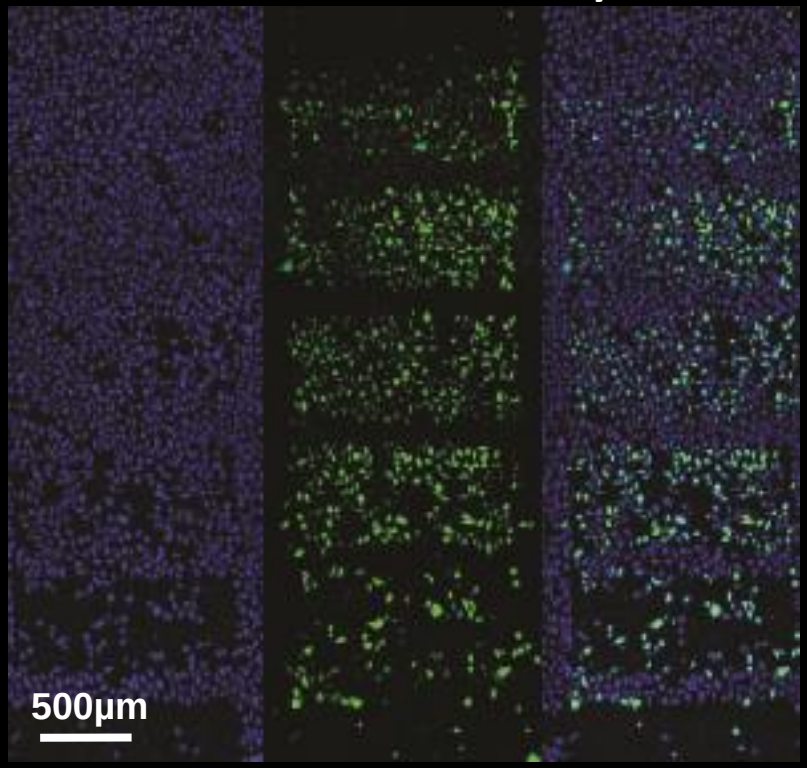
Cancer cells



Femtosecond laser



Poration and Viability



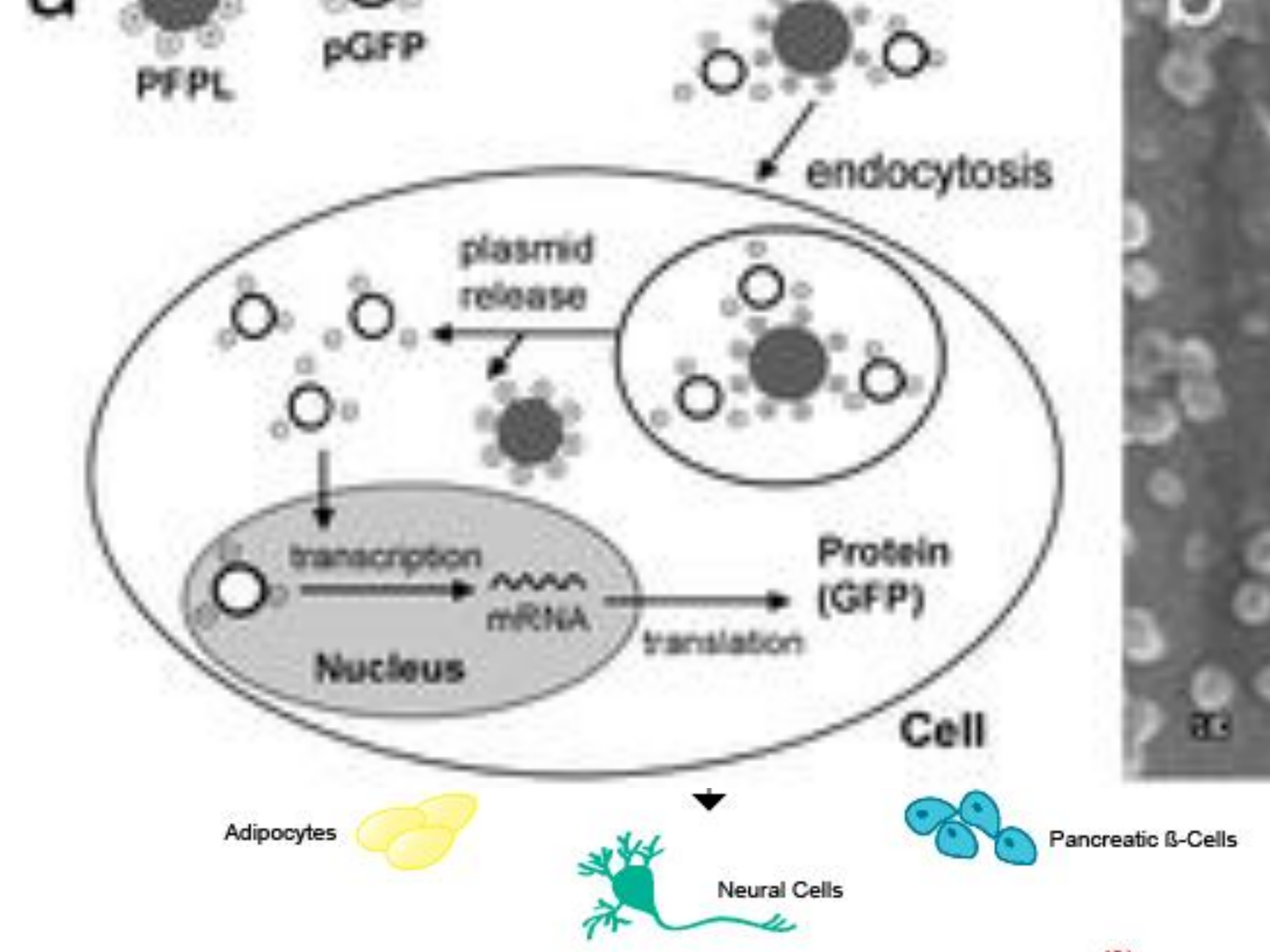
The Global Human Resource Program Bridging across Physics and Chemistry
30 January 2015, International House, Tokyo Metropolitan University (TMU), Japan

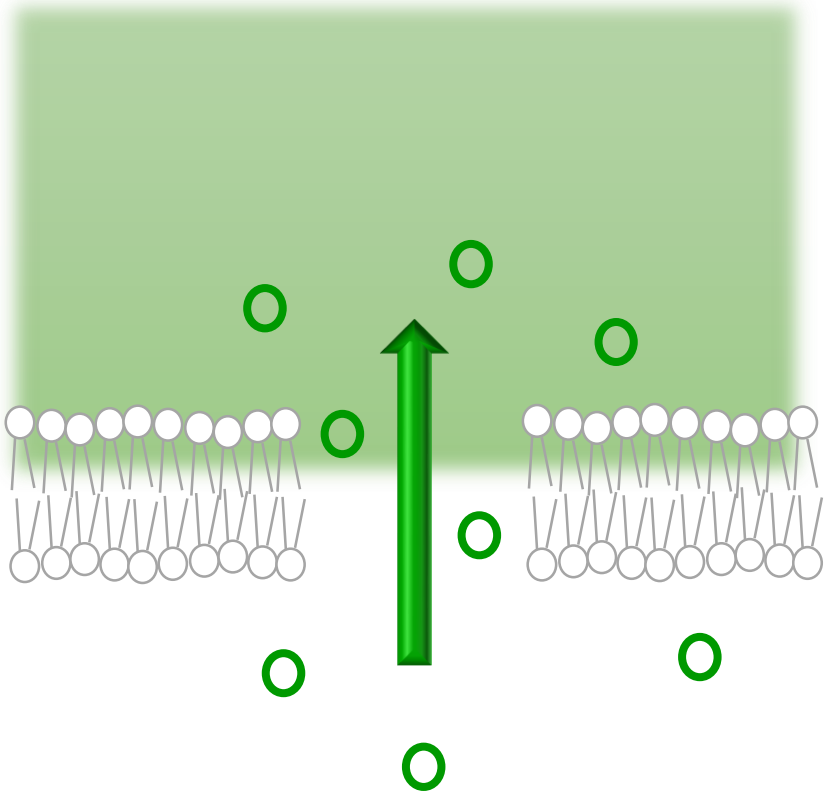
Harvard University Center for Nanoscale Systems

AAUW

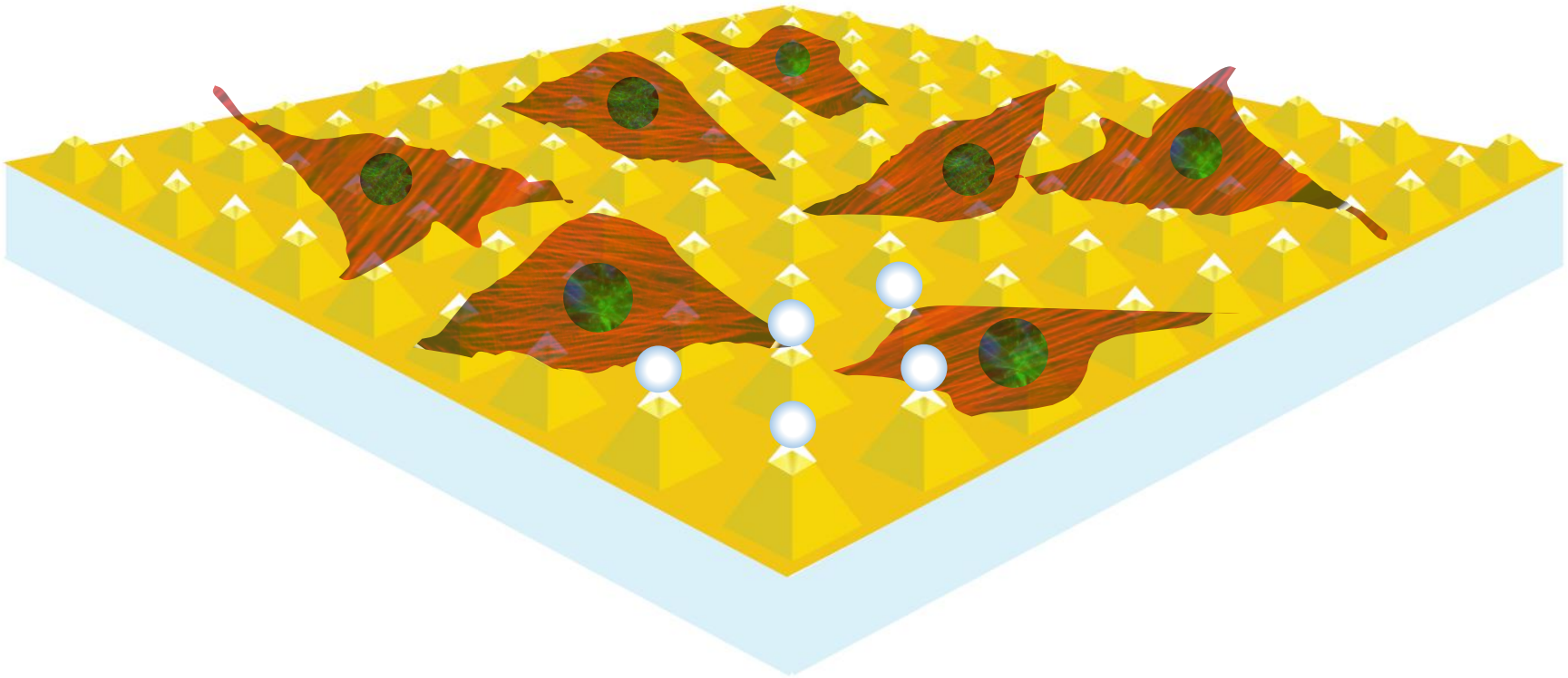
hhmi Howard Hughes Medical Institute

MAZUR GROUP

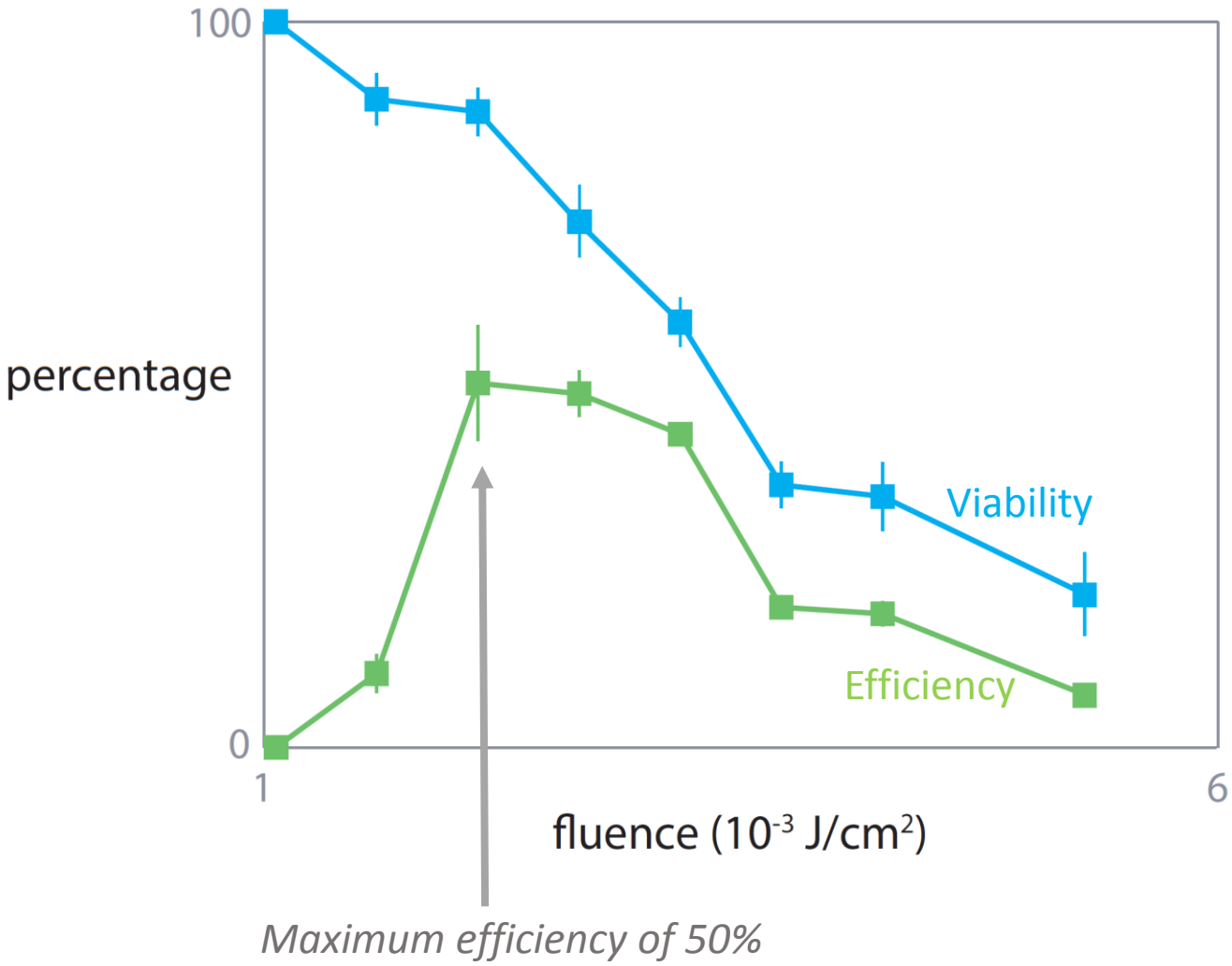

PFPL
pGFP
endocytosis
plasmid
release
transcription
mRNA
Protein
(GFP)
translation
Nucleus
Cell
Adipocytes
Neural Cells
Pancreatic ß-Cells



New approach: plasmonic pyramid substrates



many hotspots


100
percentage
0
1
6
Viability
Efficiency
fluence (10^{-3} J/cm^2)
Maximum efficiency of 50%