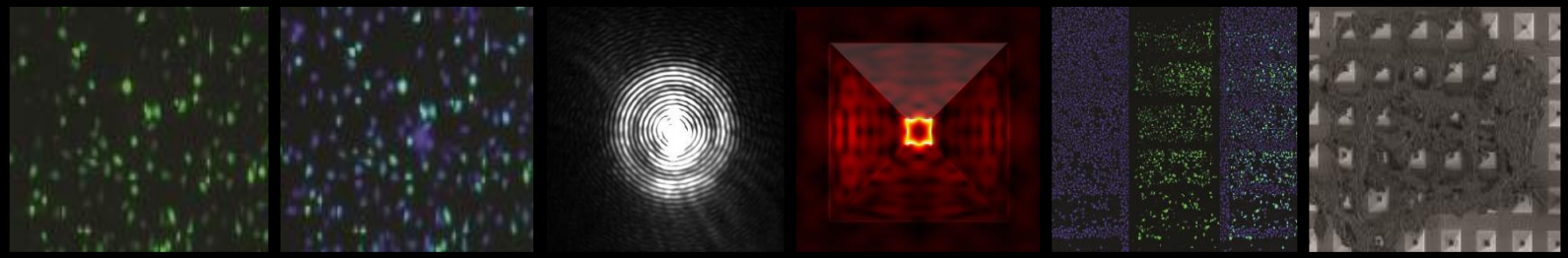


Plasmonic cell transfection using micropyramid arrays

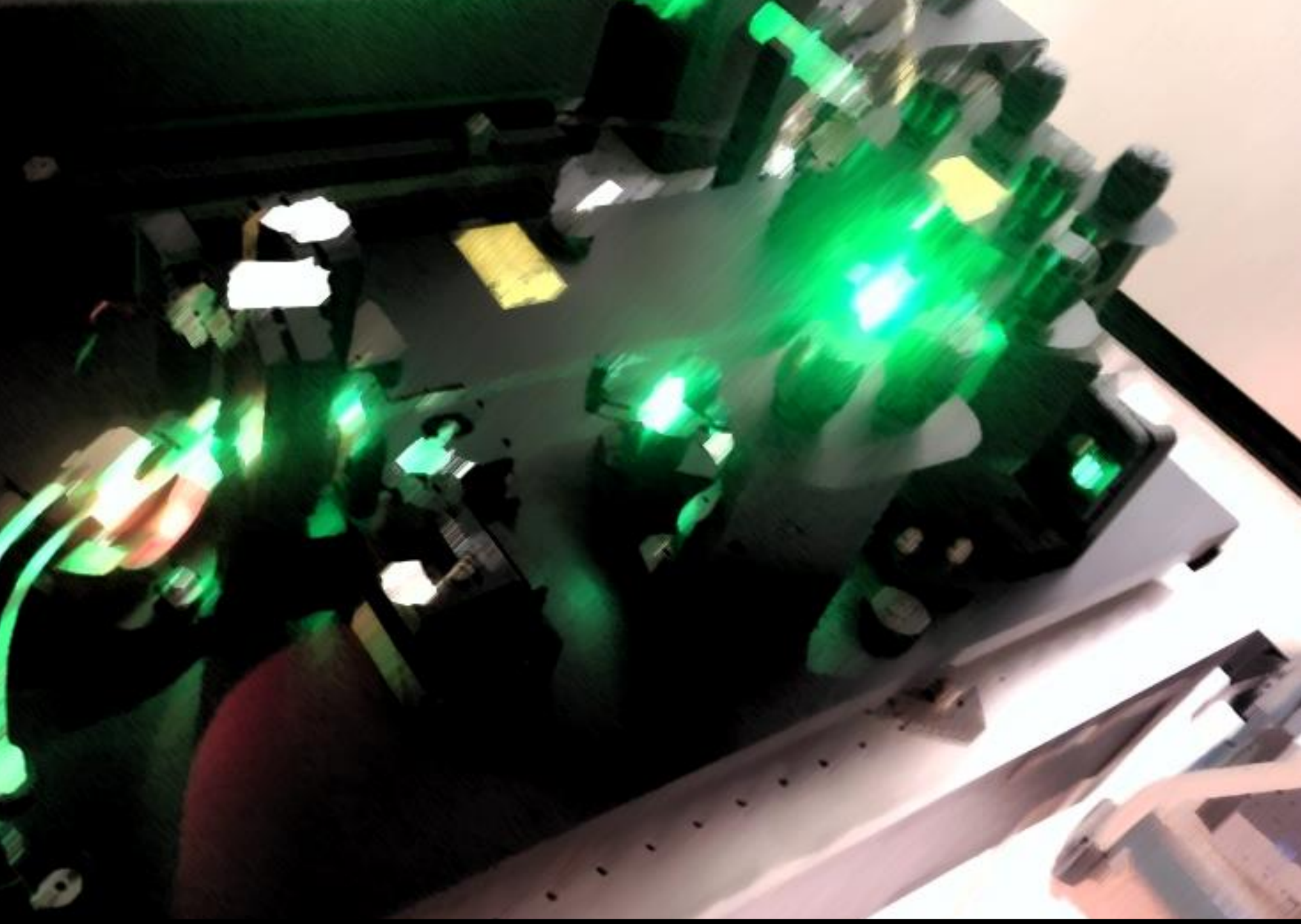
Nabiha Saklayen

M. Huber, D. I. Vulis, M. Madrid, L. Milling, S. Courvoisier, V. Nuzzo, E. Mazur

Mazur Group
Department of Physics and School of Engineering and Applied Sciences
Harvard University

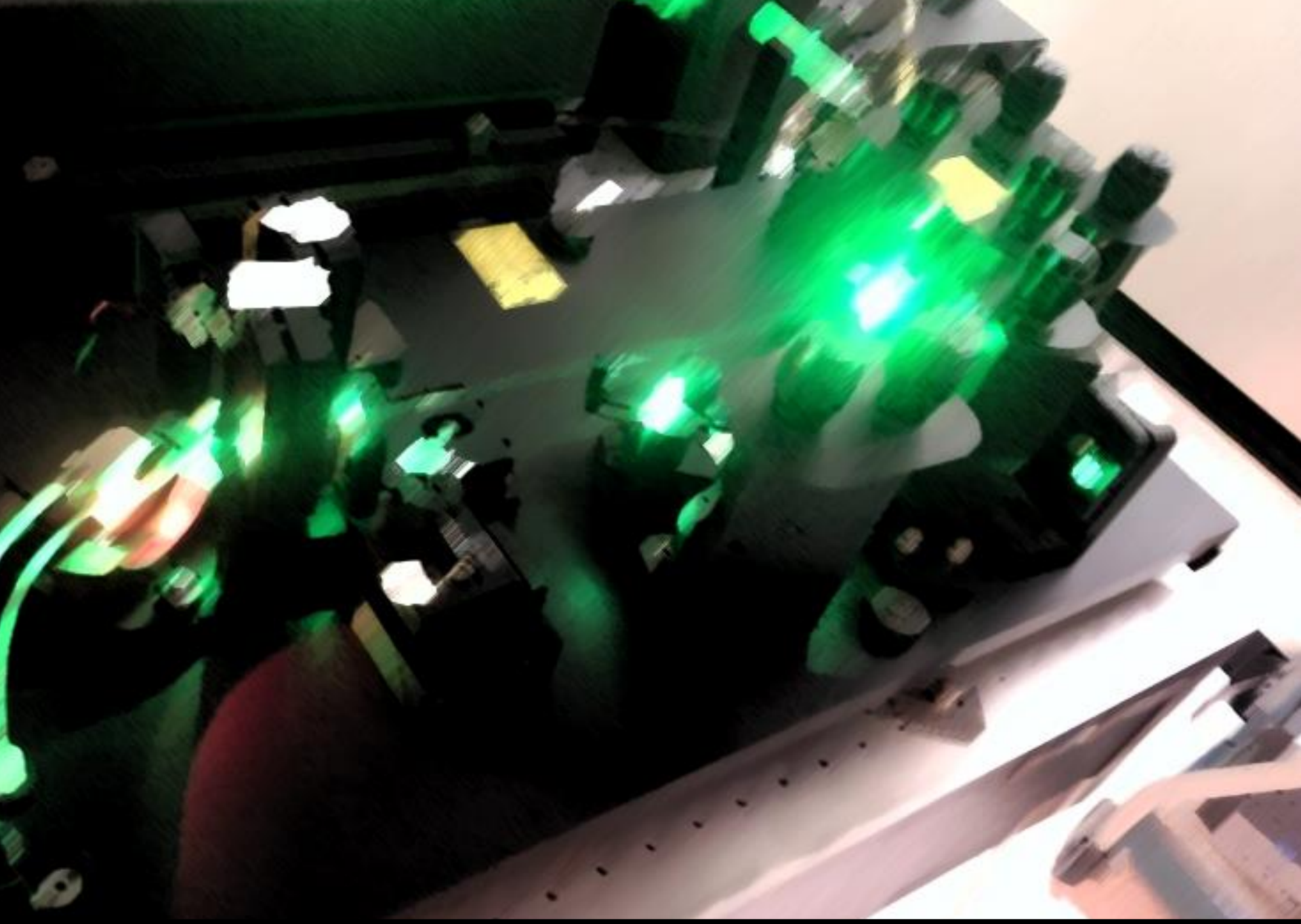
Photonics West, 8 February 2015

1 Introduction



1 Introduction

2 Experiments

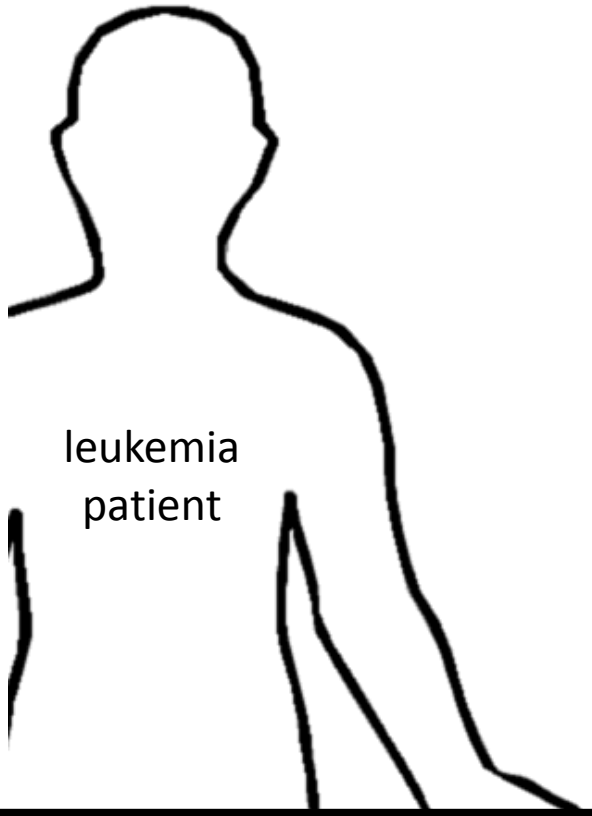


1 Introduction

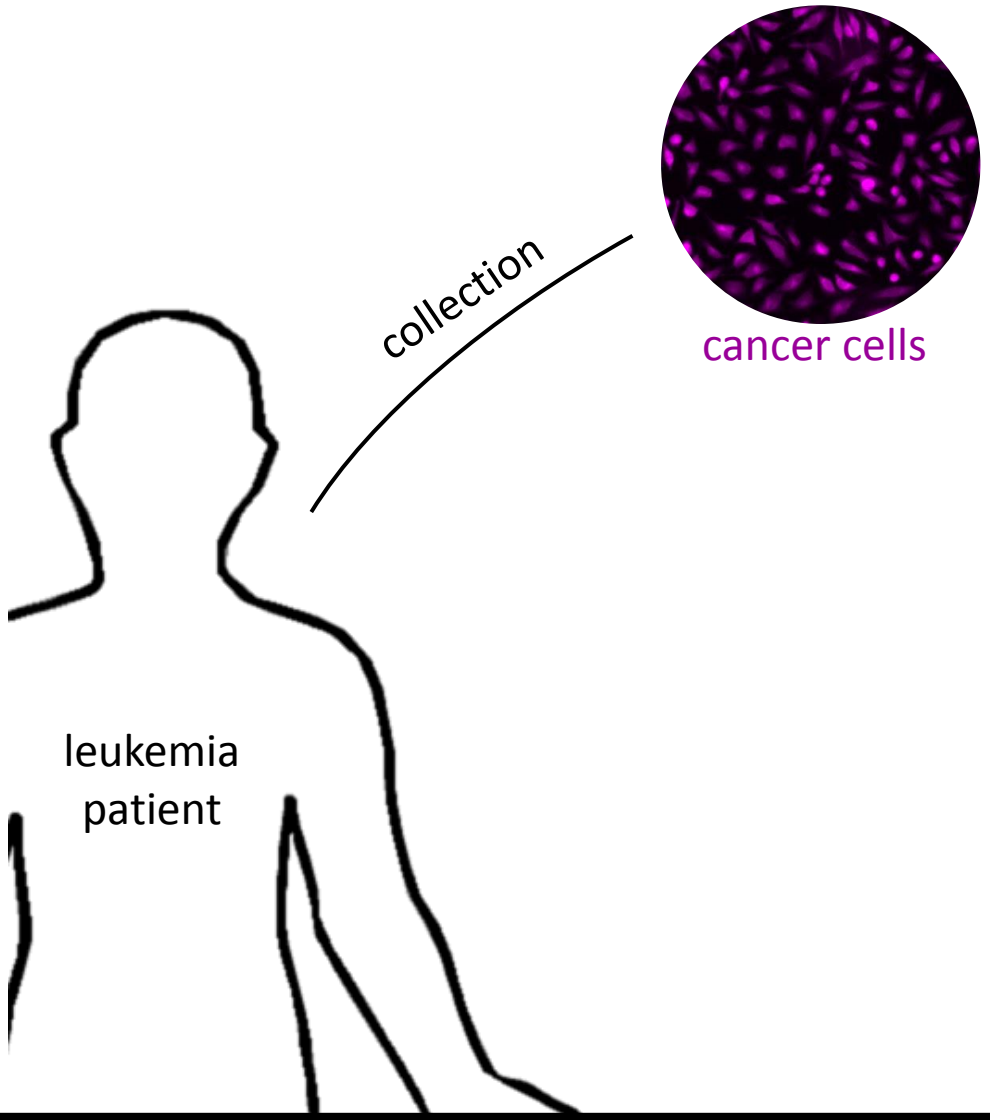
2 Experiments

3 Results

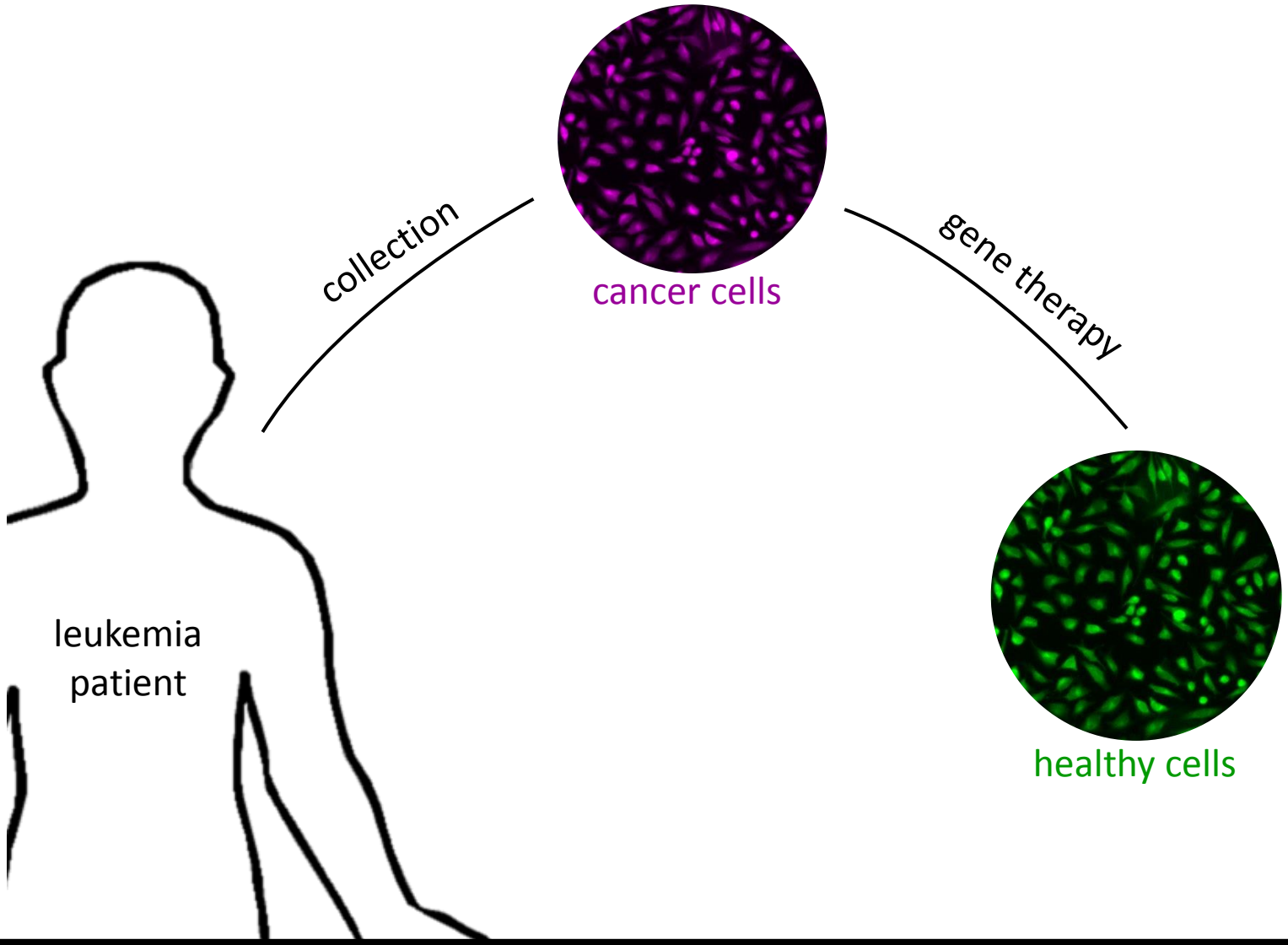
The future: medicine based on our genes



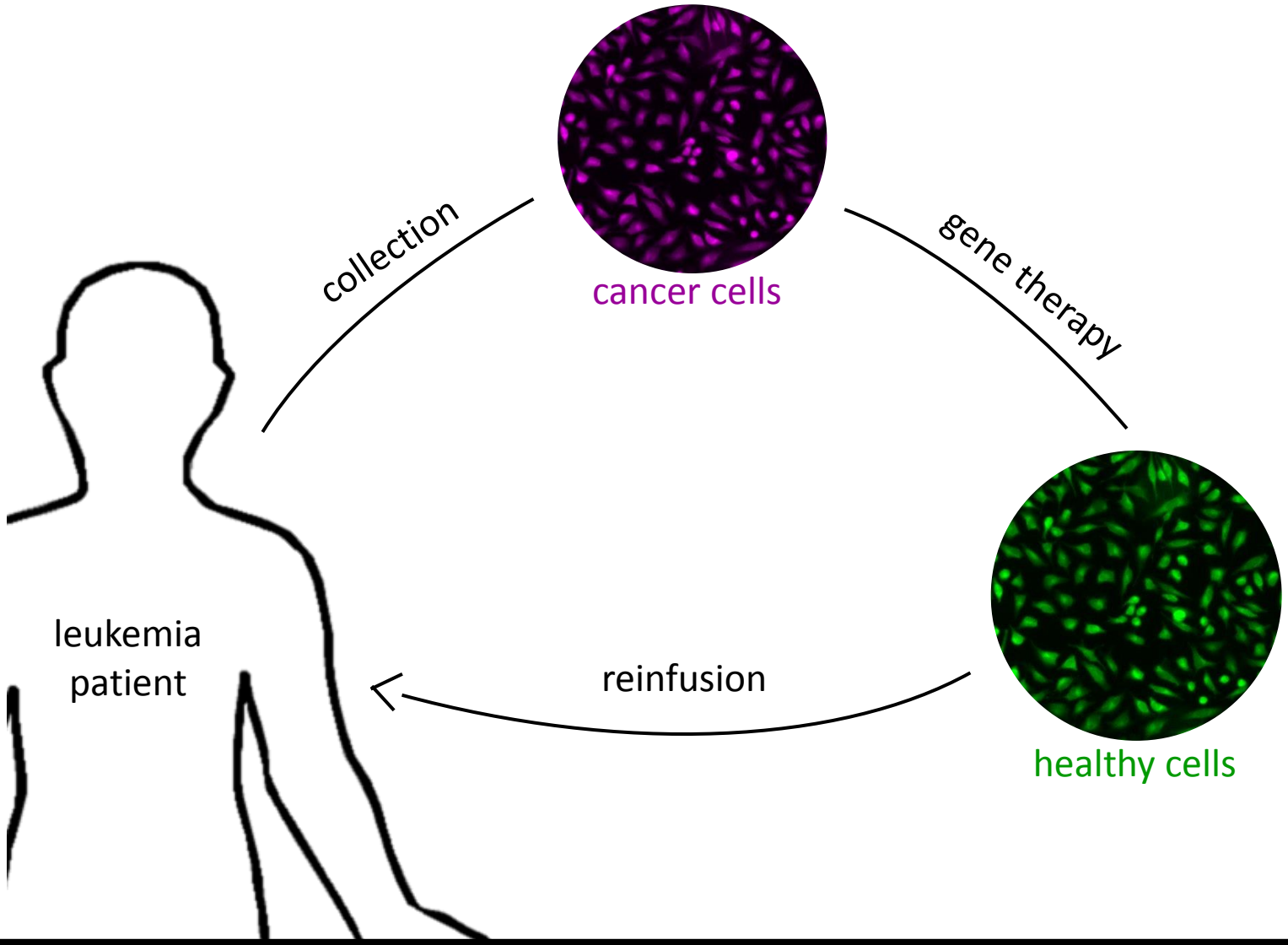
The future: medicine based on our genes



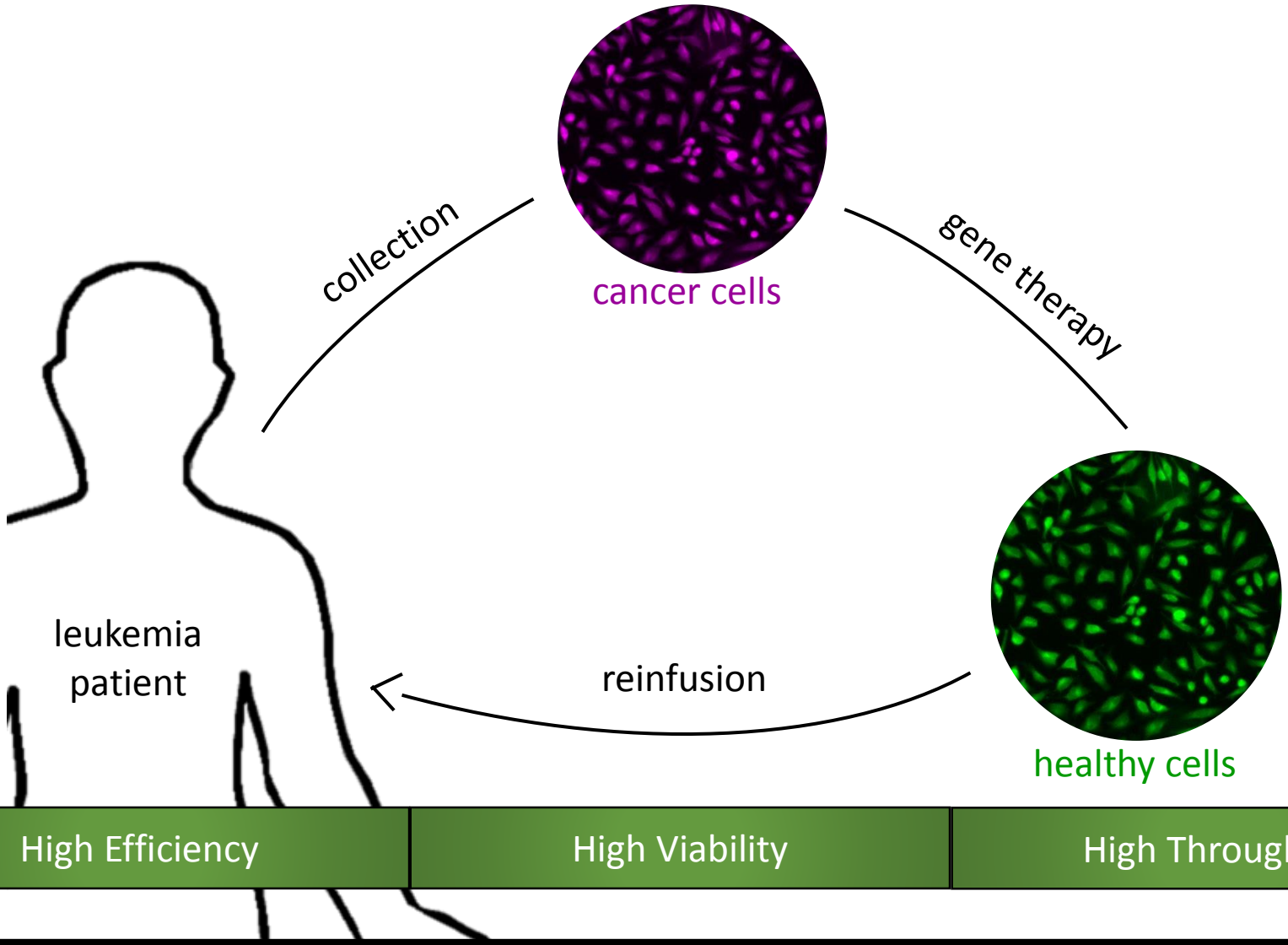
The future: medicine based on our genes



The future: medicine based on our genes

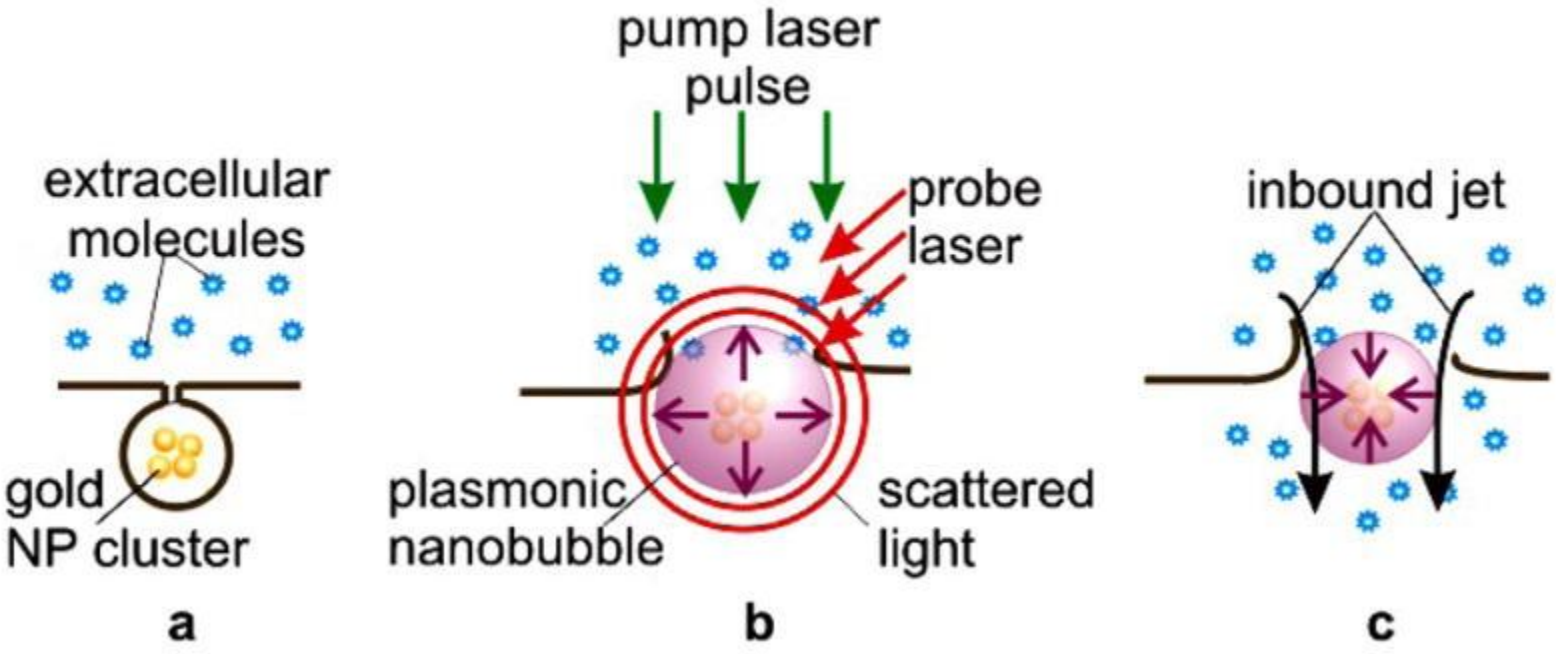


The future: medicine based on our genes



High Efficiency | High Viability | High Throughput

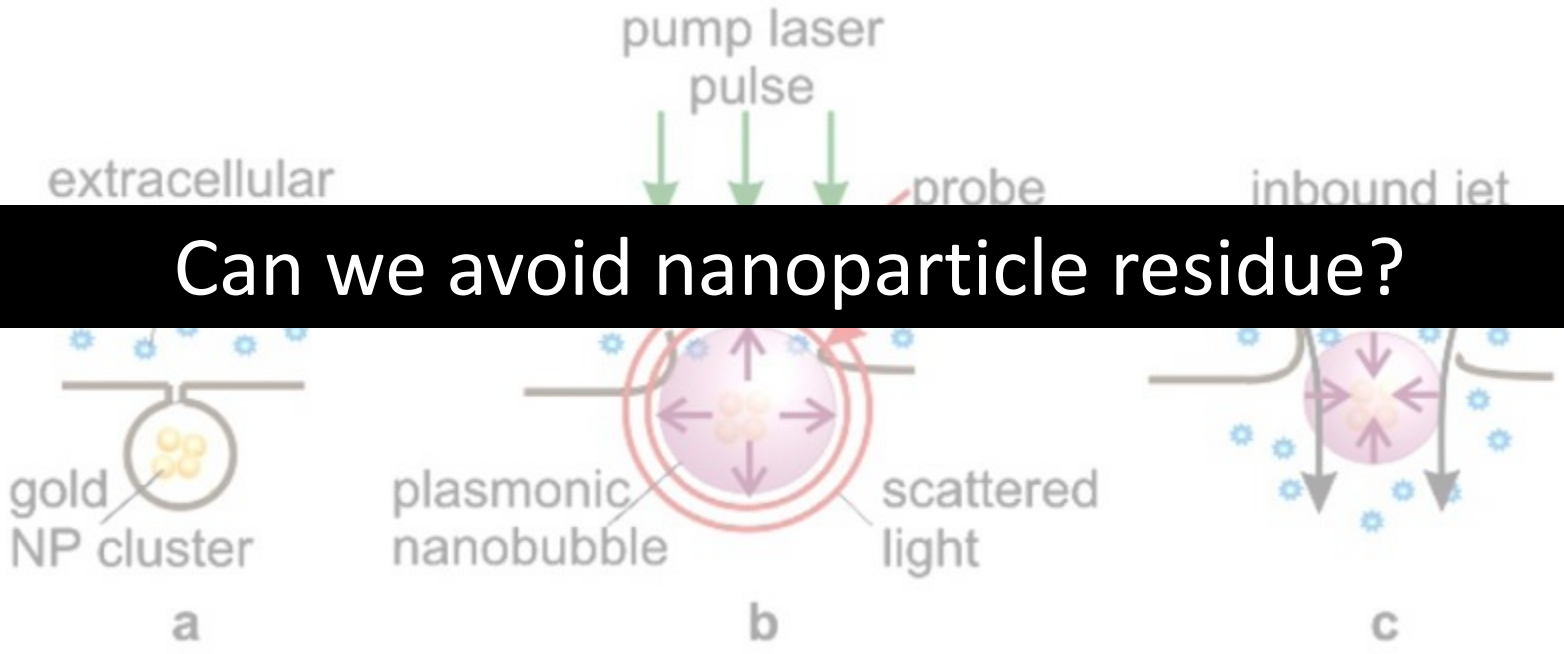
Plasmonic nanoparticle transfection offers desirable features



Lukianova-Hleb, Ekaterina Y., et al. "Selective gene transfection of individual cells in vitro with plasmonic nanobubbles." *Journal of Controlled Release* 152.2 (2011): 286-293.

High Efficiency	High Viability	High Throughput

Plasmonic nanoparticle transfection offers desirable features

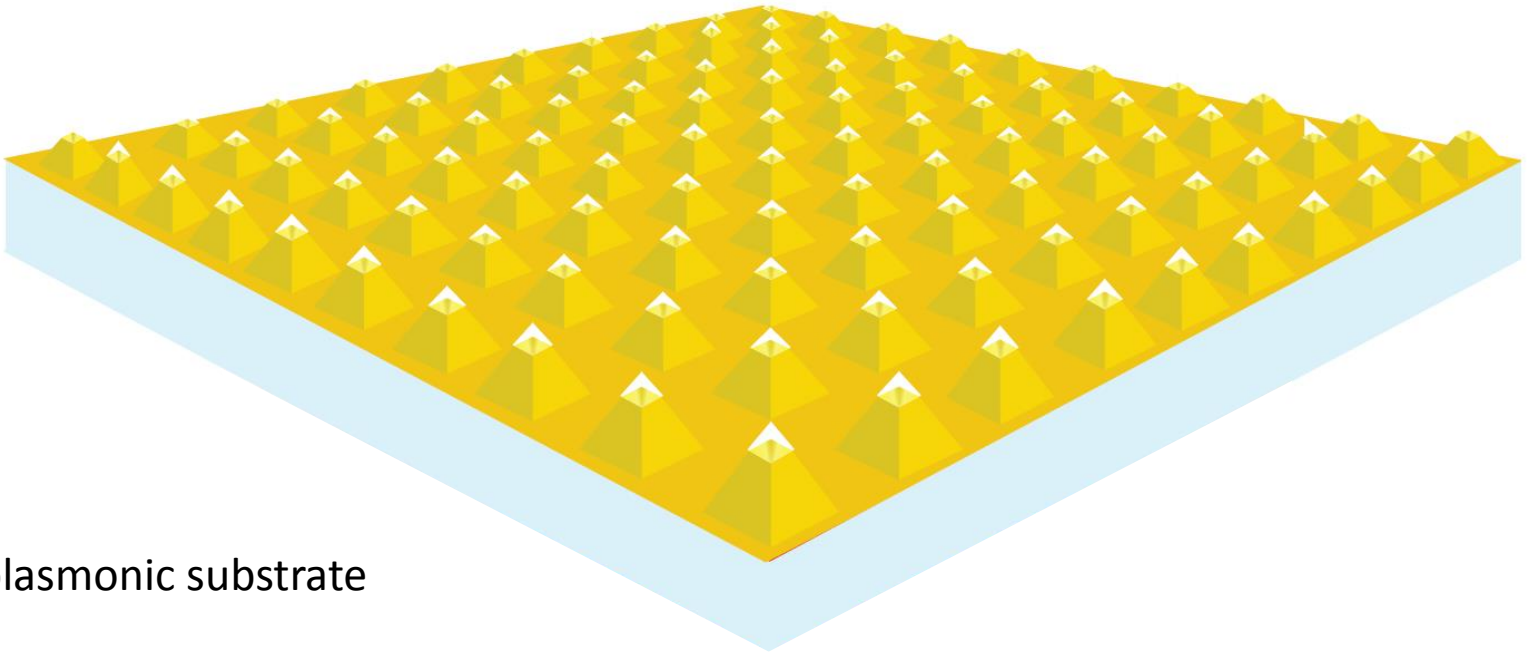


Can we avoid nanoparticle residue?

Lukianova-Hleb, Ekaterina Y., et al. "Selective gene transfection of individual cells in vitro with plasmonic nanobubbles." *Journal of Controlled Release* 152.2 (2011): 286-293.

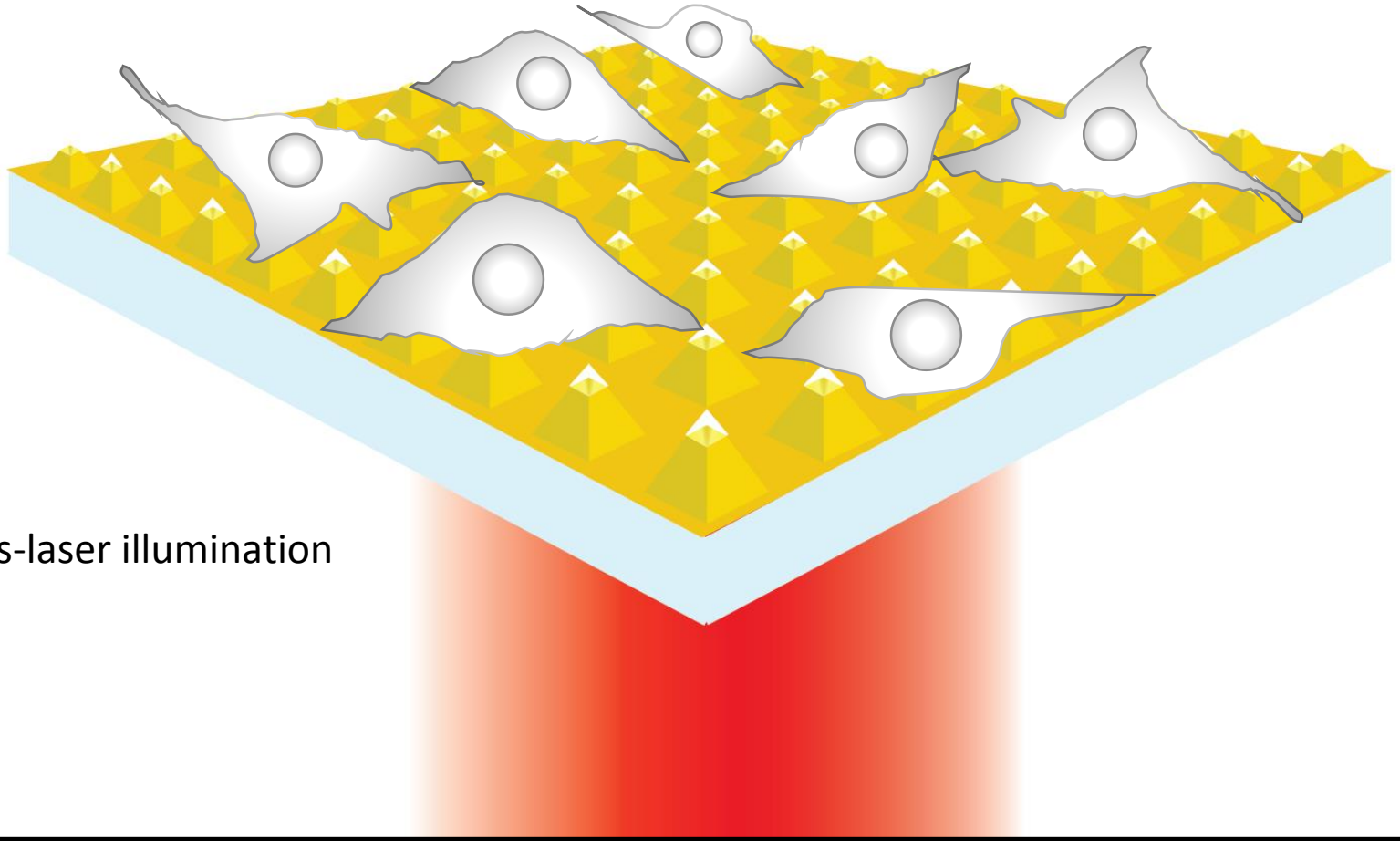
High Efficiency	High Viability	High Throughput

Plasmonic substrates for cell transfection



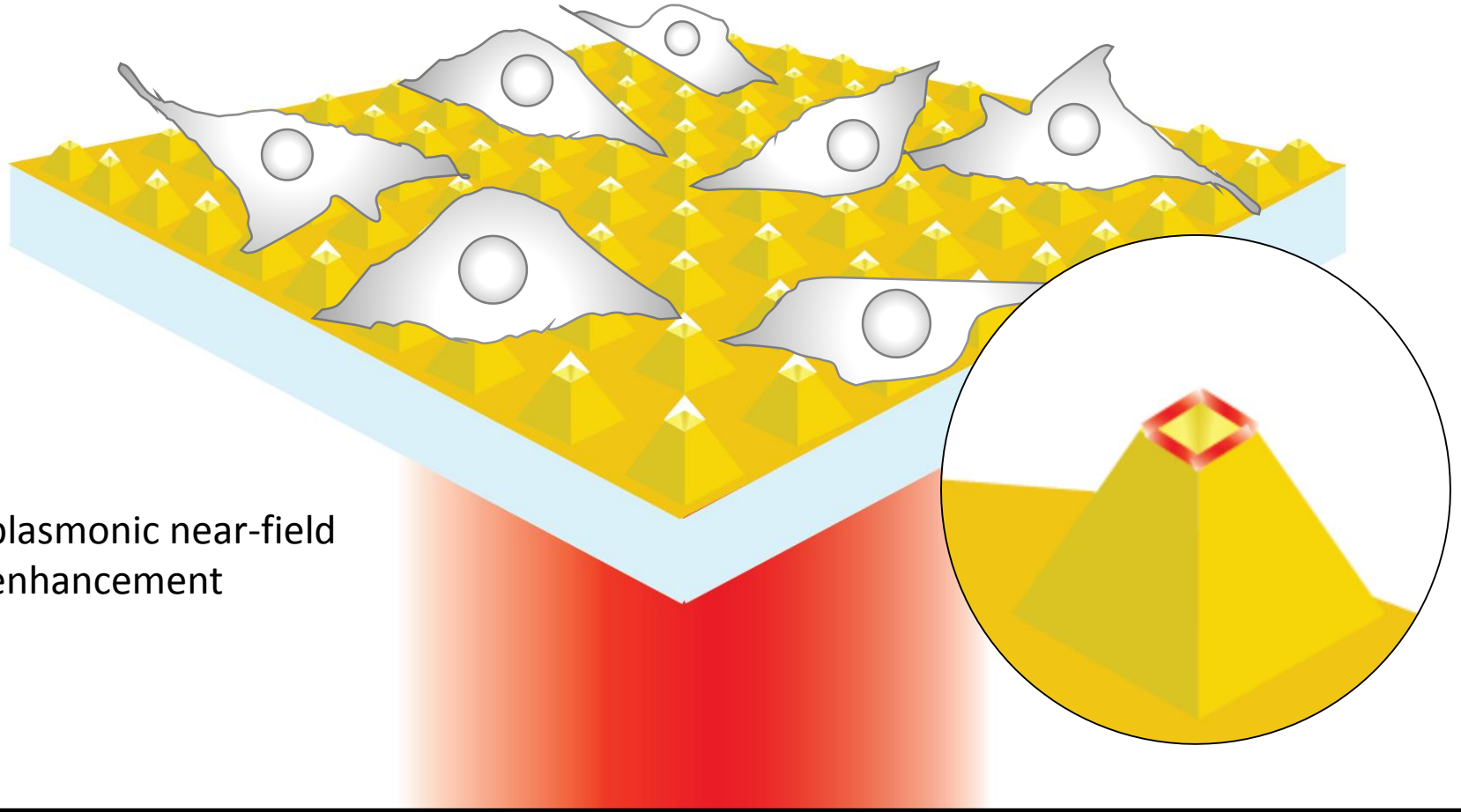
plasmonic substrate

Plasmonic substrates for cell transfection



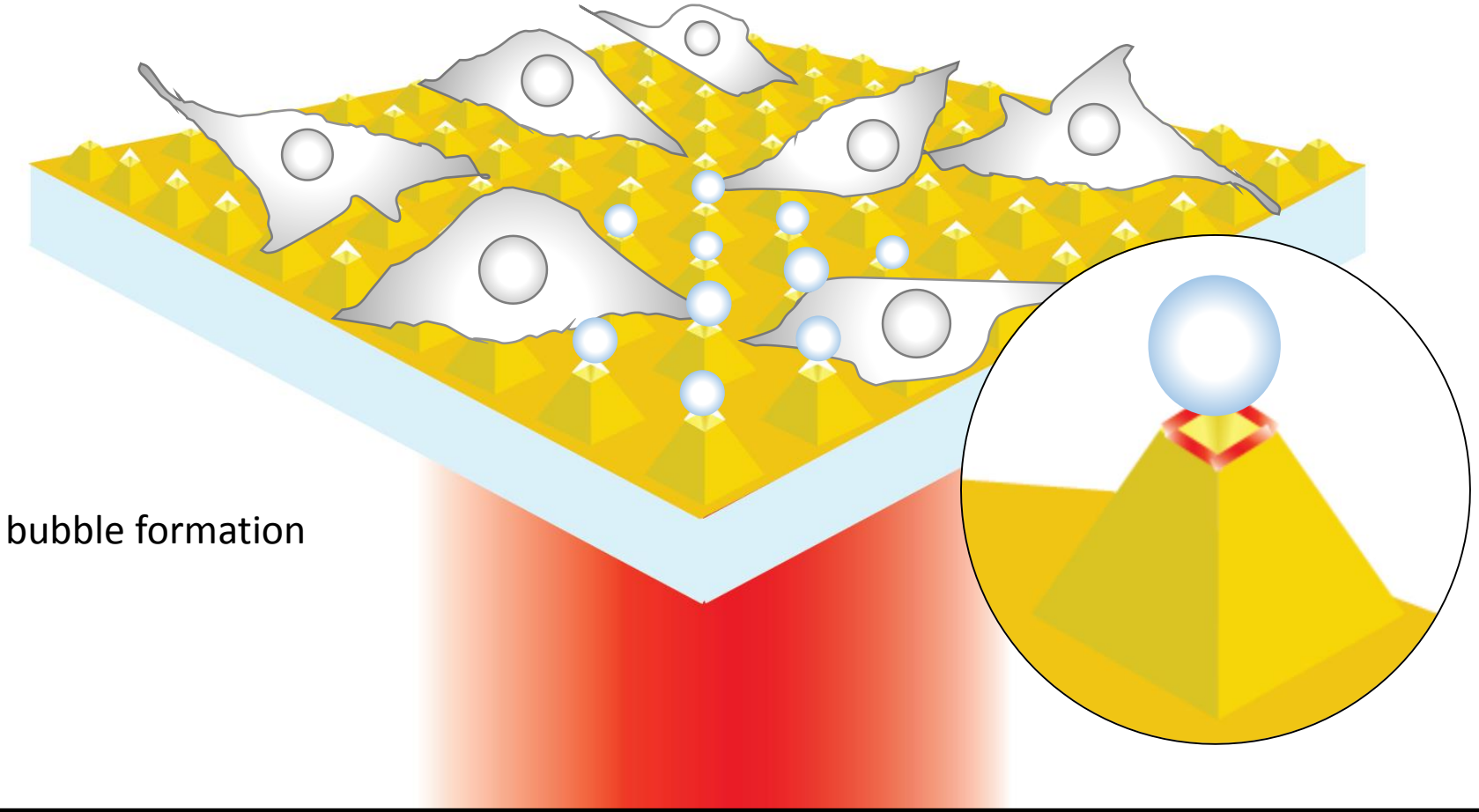
fs-laser illumination

Plasmonic substrates for cell transfection



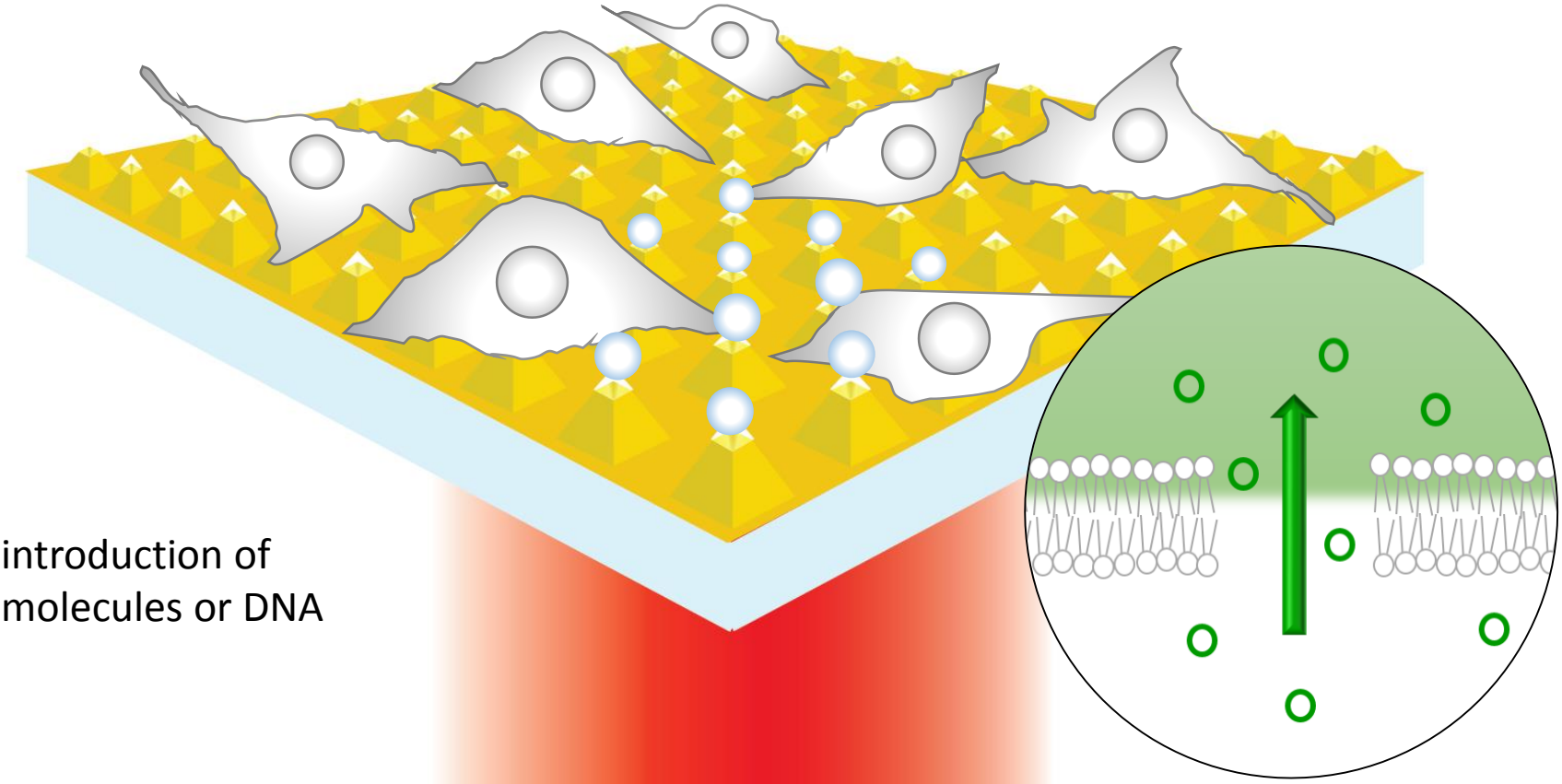
plasmonic near-field enhancement

Plasmonic substrates for cell transfection



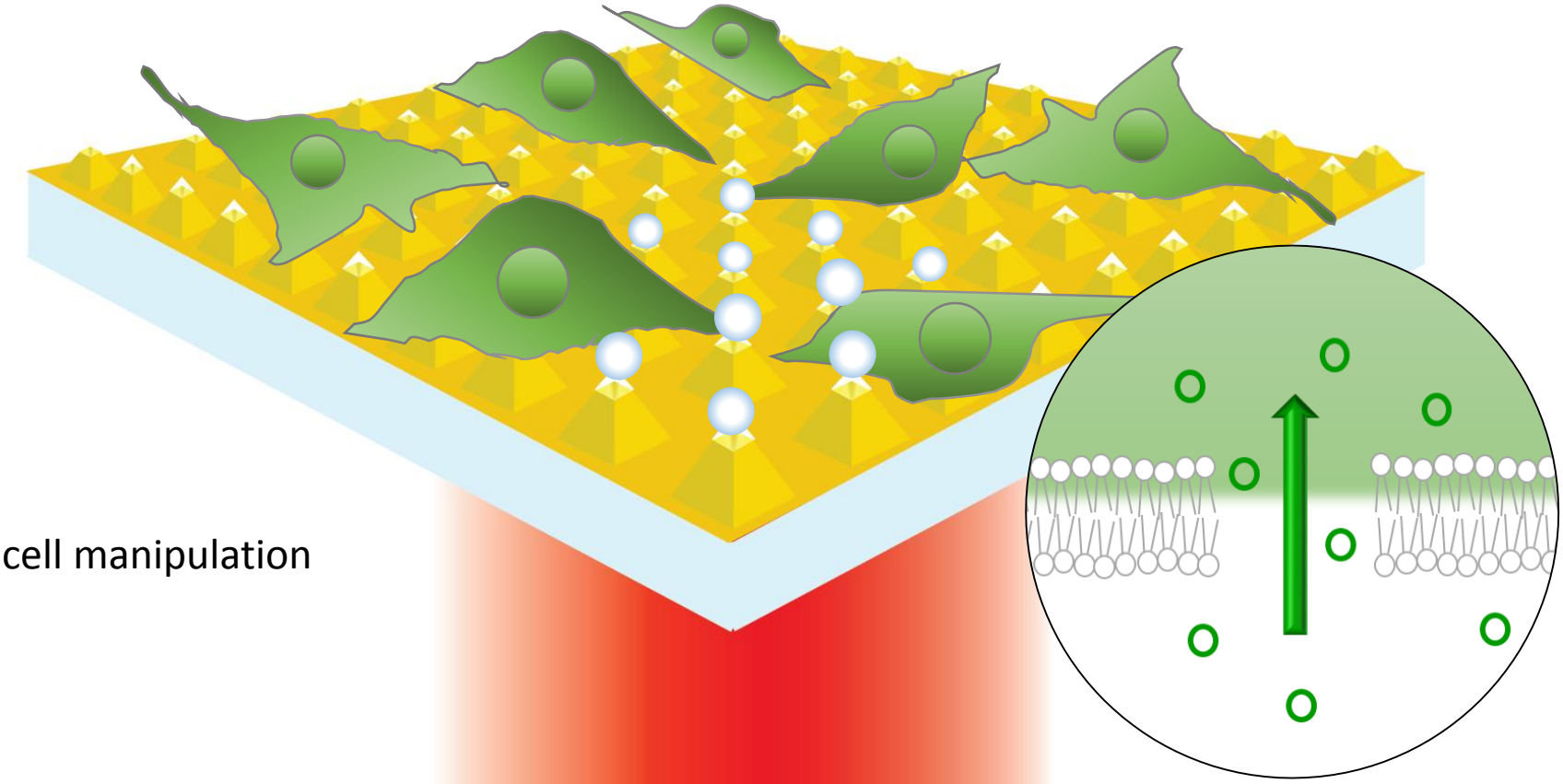
bubble formation

Plasmonic substrates for cell transfection



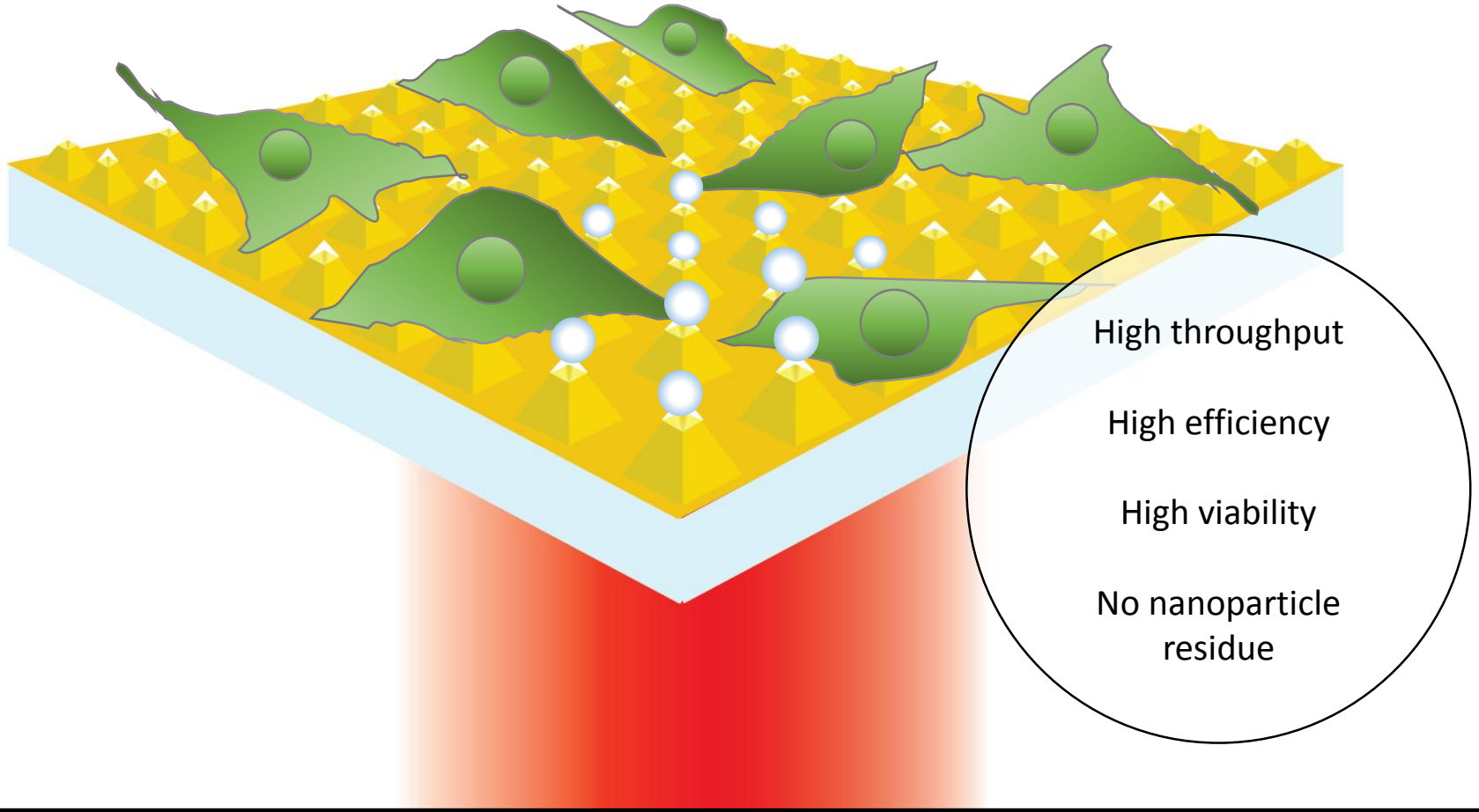
introduction of
molecules or DNA

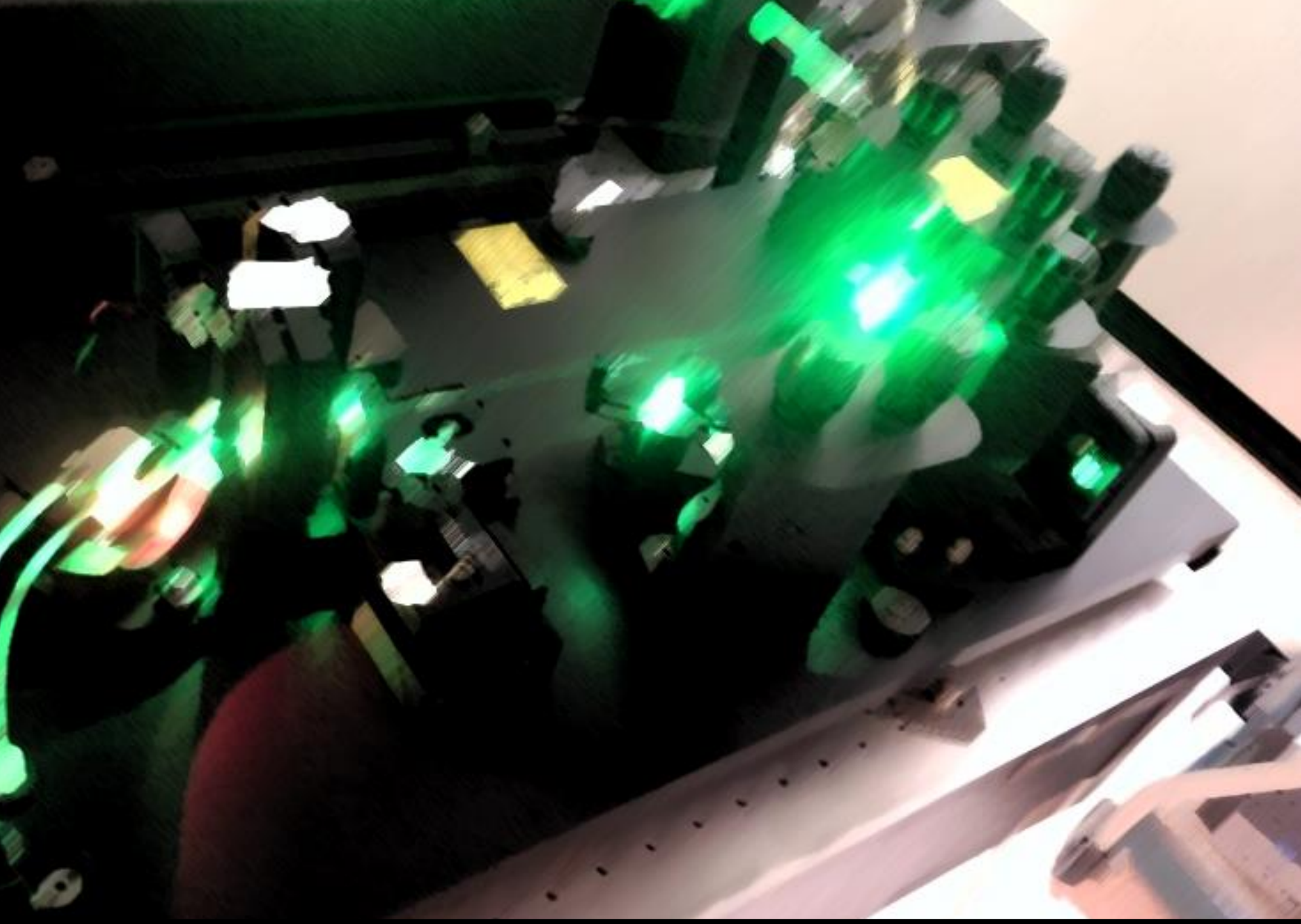
Plasmonic substrates for cell transfection



cell manipulation

Plasmonic substrates for cell transfection

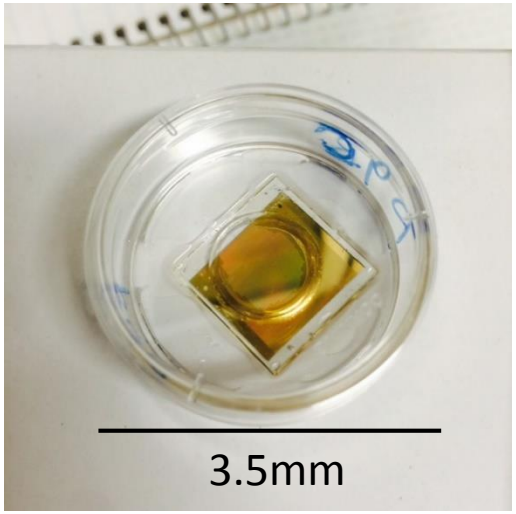




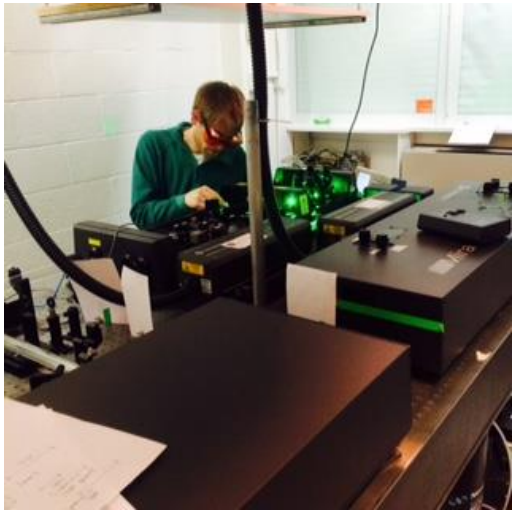
1 Introduction

2 Experiments

Poration experiments have three components



Cell culture



Laser scanning

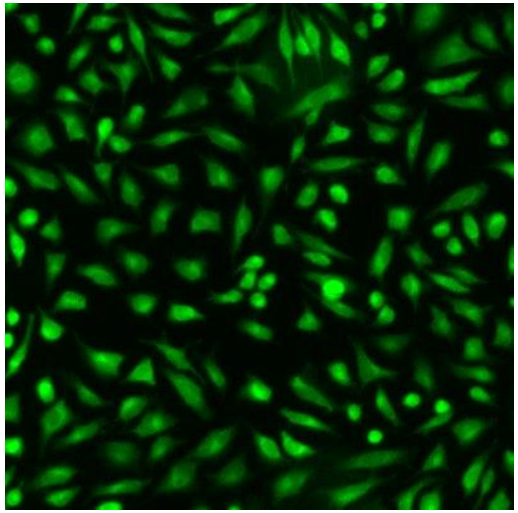
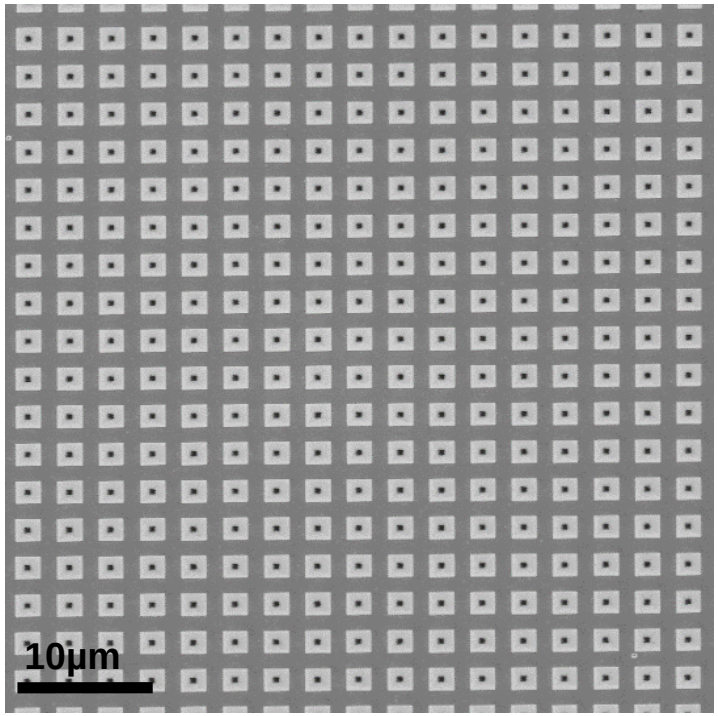
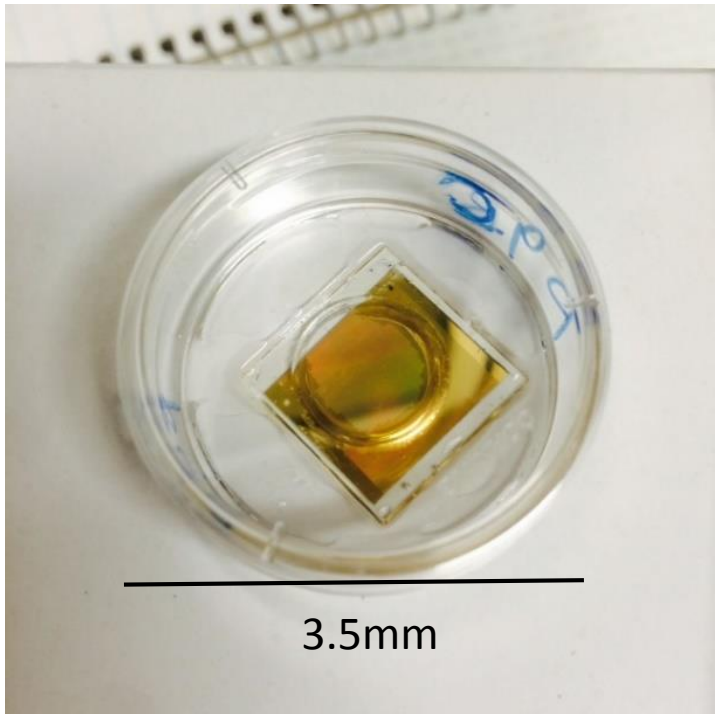


Image analysis

Plasmonic substrates attached to petri dishes

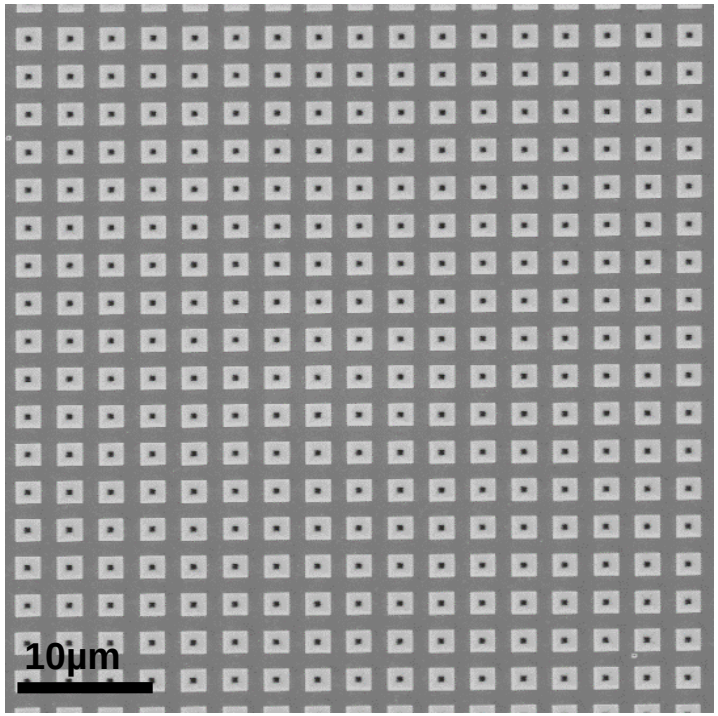


photolithography

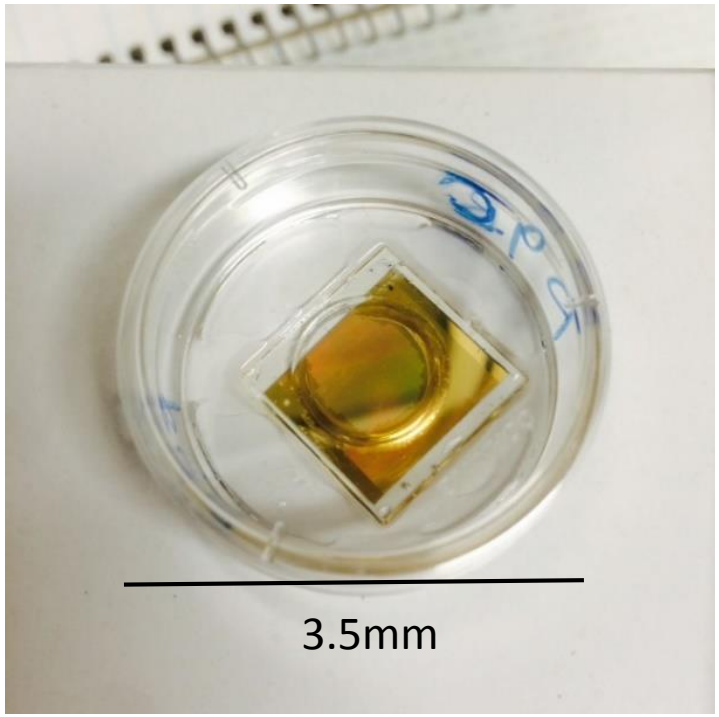


petri dishes

Plasmonic substrates attached to petri dishes



photolithography

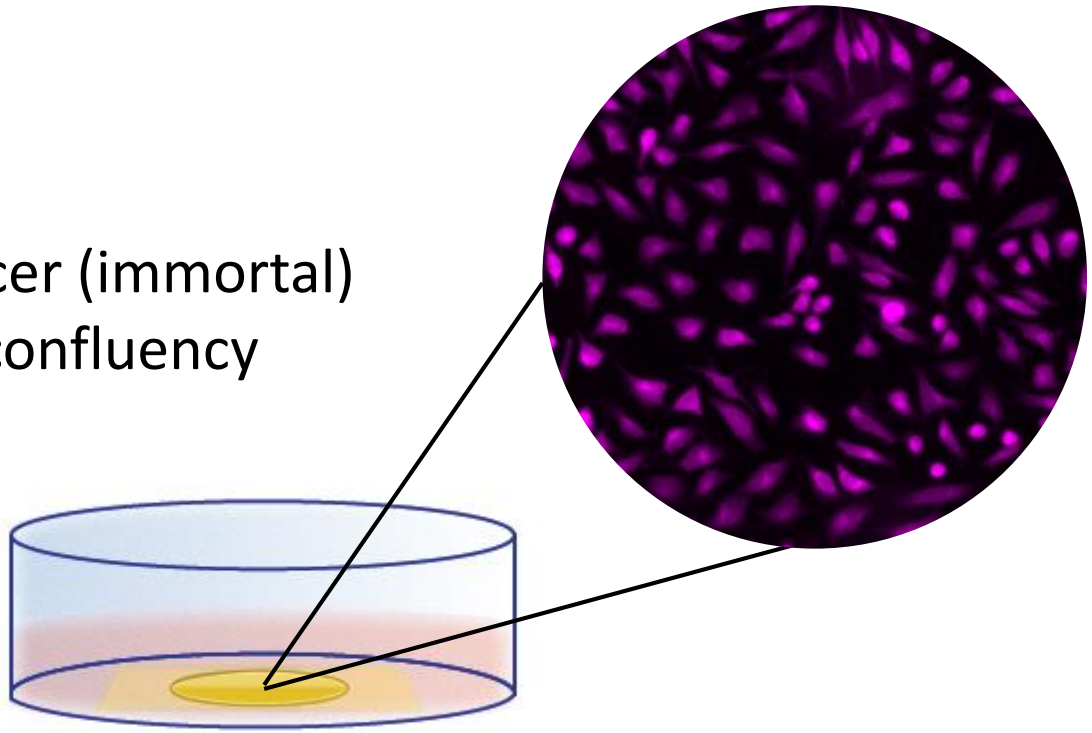


petri dishes

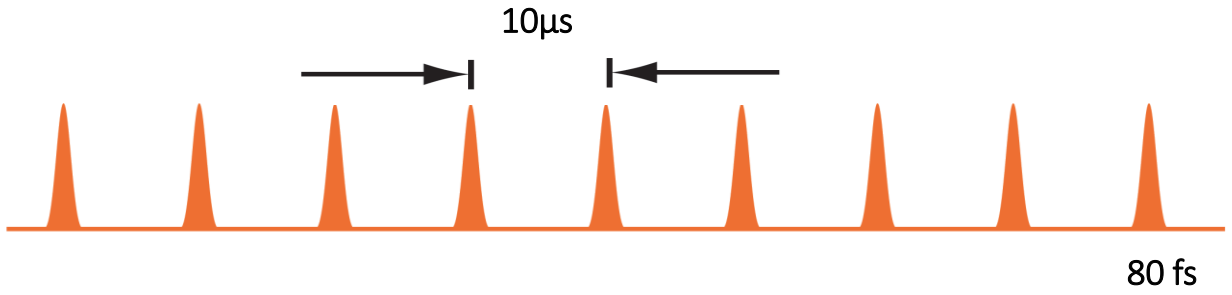
Design, simulation, and fabrication of plasmonic pyramid substrate for cell transfection (Paper 9355-16)
Time: 5:40 PM - 6:00 PM, Marinus Huber

Cell culture on plasmonic substrates

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



Ti: sapphire laser scanning for poration

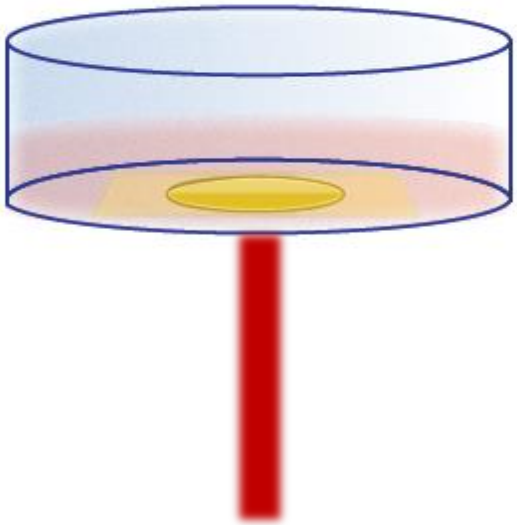


rep rate: 100 kHz

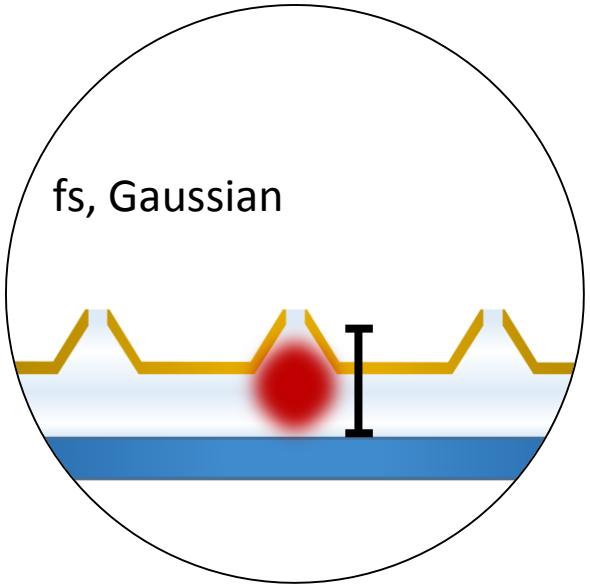
energy per pulse: 3µJ

peak power: 10^6 W

wavelength: 800 nm

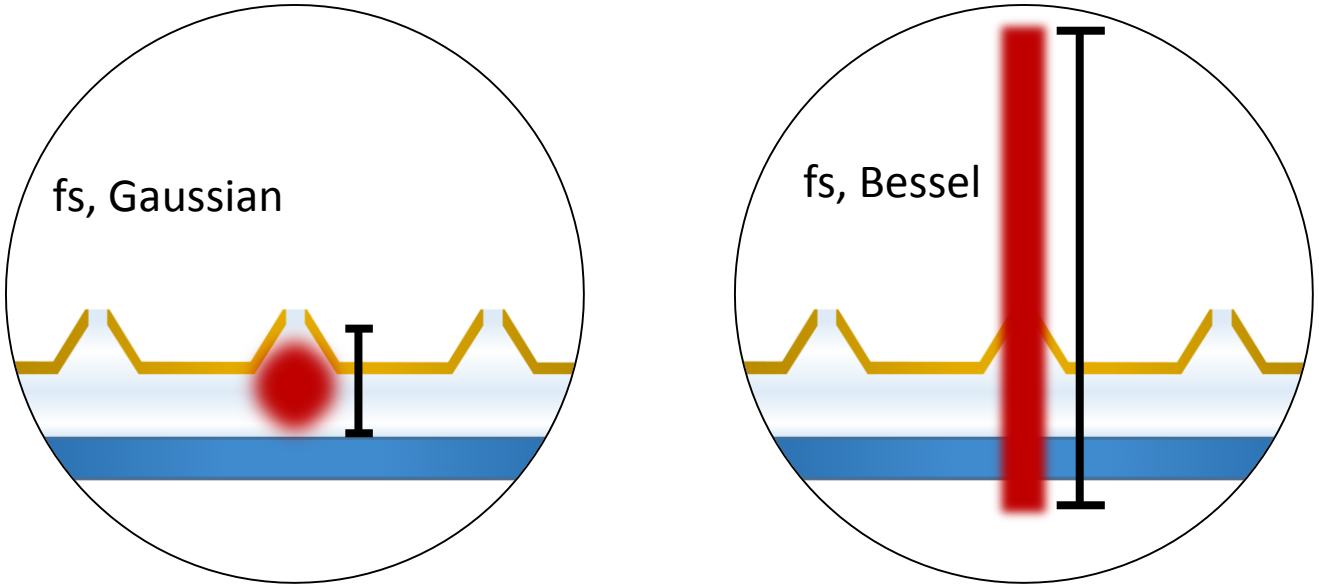


Laser spot needs to hit the sample



Suitable for scanning smaller regions

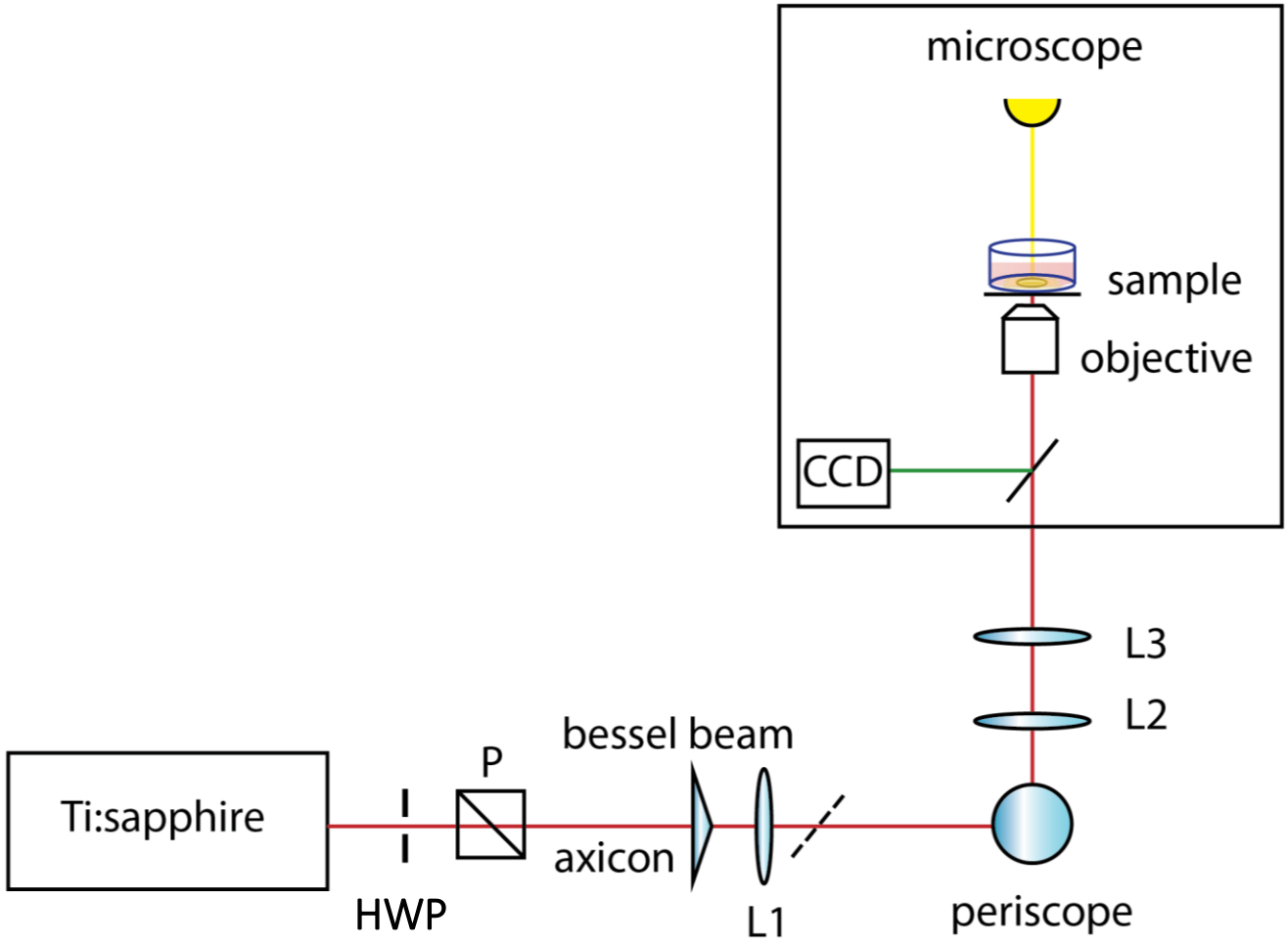
A non-diffracting light beam allows us to scan large areas



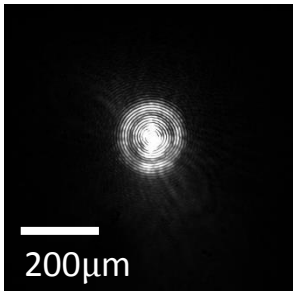
Can scan very large areas

Tsampoula, Xanthi, et al. "Femtosecond cellular transfection using a nondiffracting light beam." *Applied Physics Letters* 91.5 (2007): 053902.

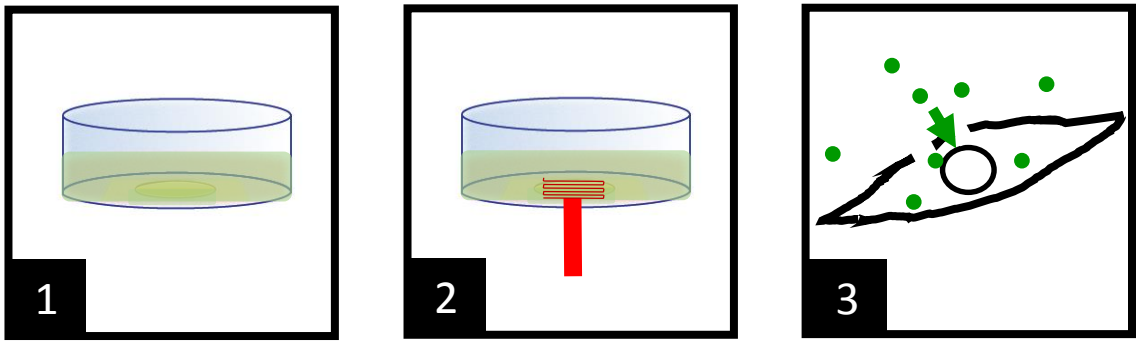
A non-diffracting light beam allows us to scan large areas



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

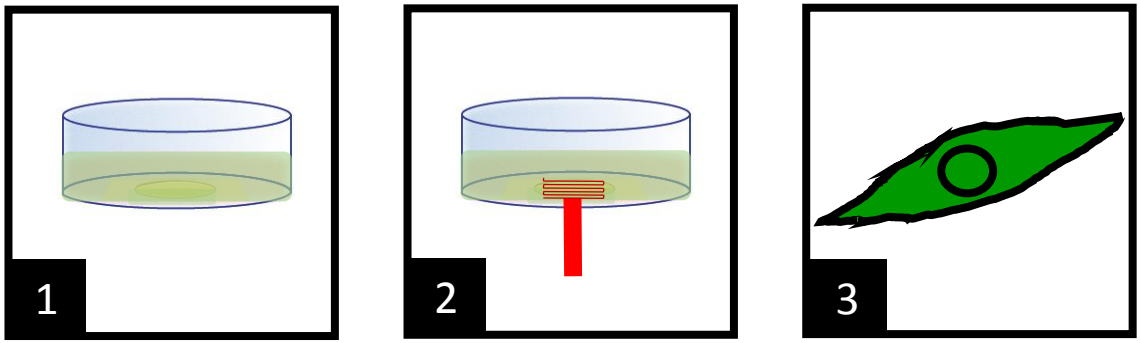


Experimental procedure



Add dye molecules | Laser scanning | Poration

Experimental procedure

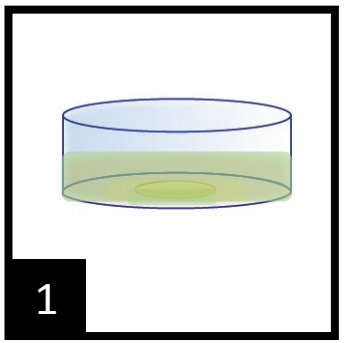


Add dye molecules

Laser scanning

Poration

Experimental procedure



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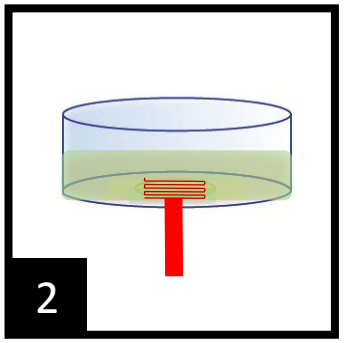
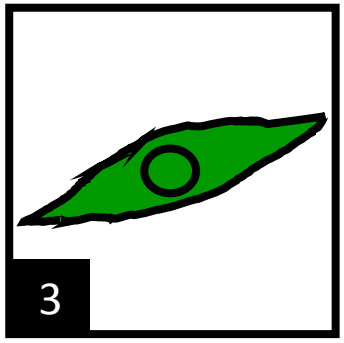
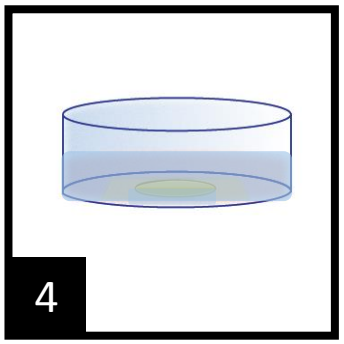


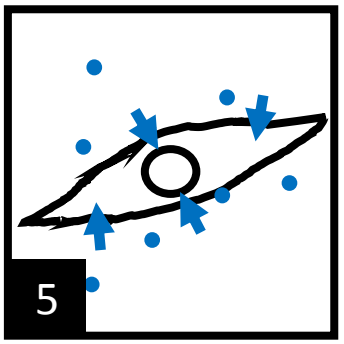
Laser scanning



Poration

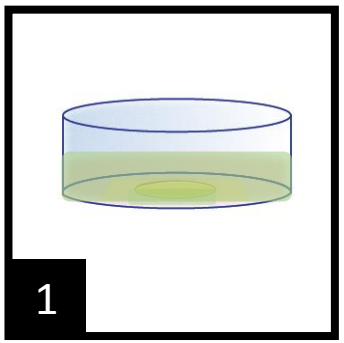


Calcein AM uptake

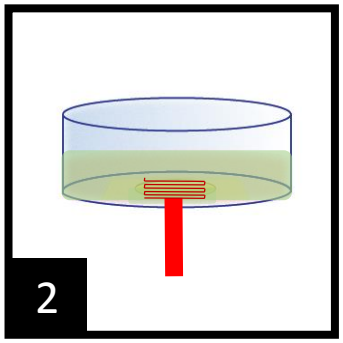


Viability

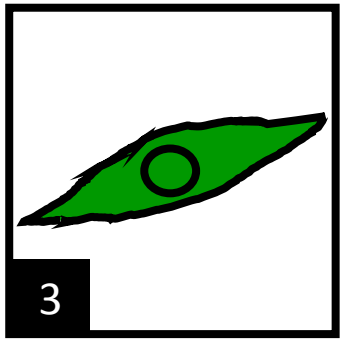
Experimental procedure



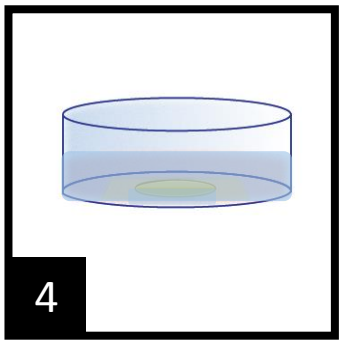
Add dye molecules



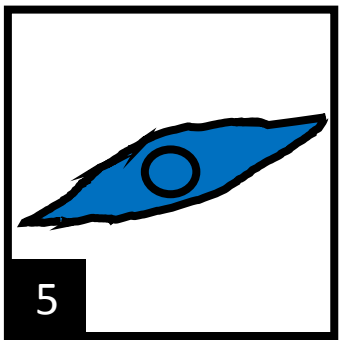
Laser scanning



Poration

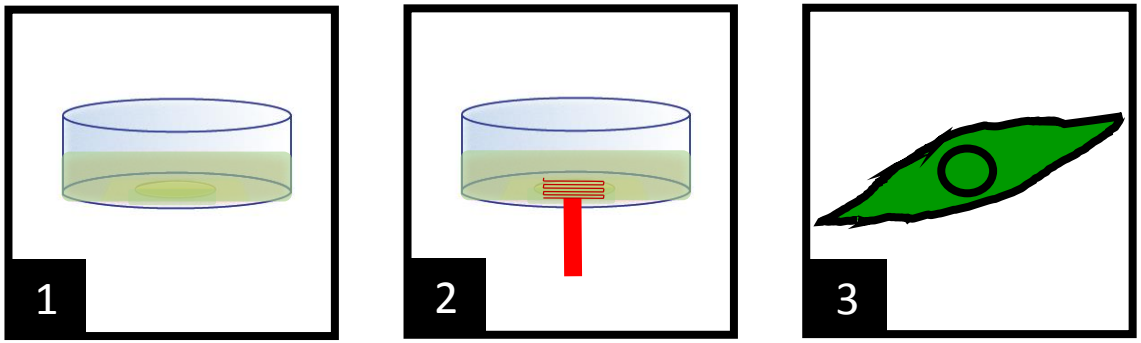


Calcein AM uptake

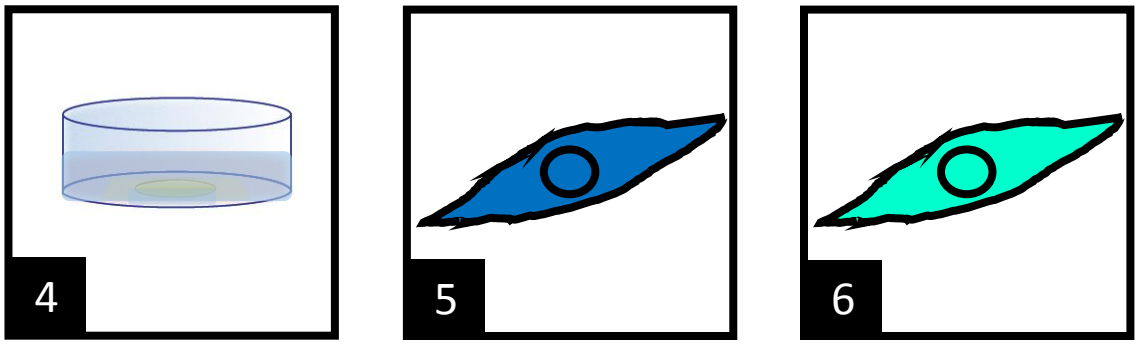


Viability

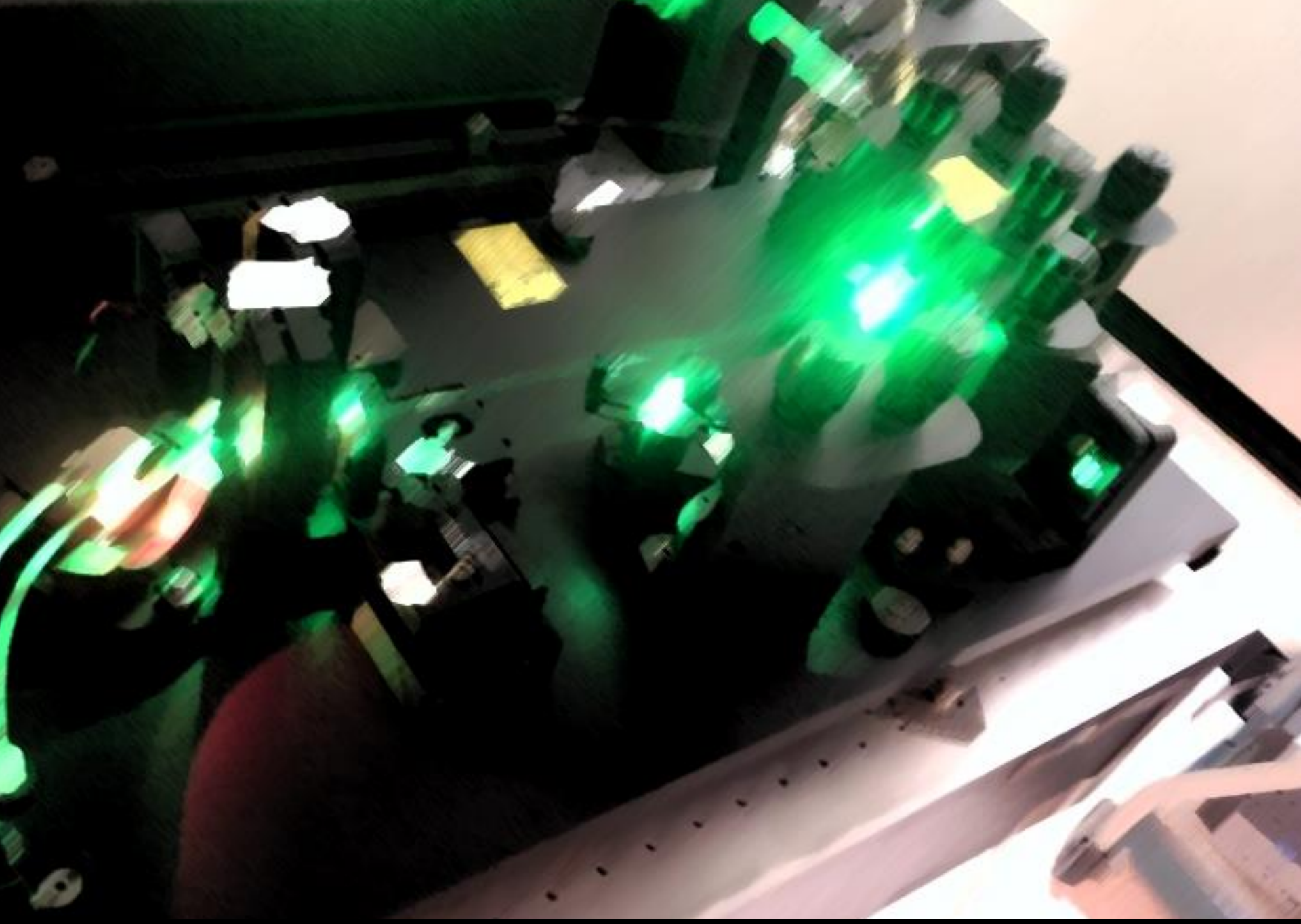
Experimental procedure



Add dye molecules | Laser scanning | Poration



Calcein AM uptake | Viability | Poration and Viability



1 Introduction

2 Experiments

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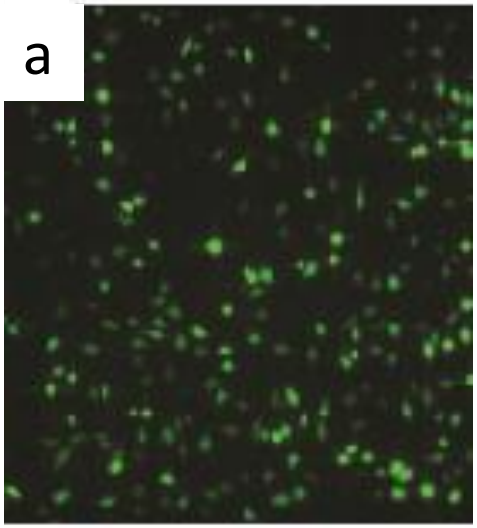
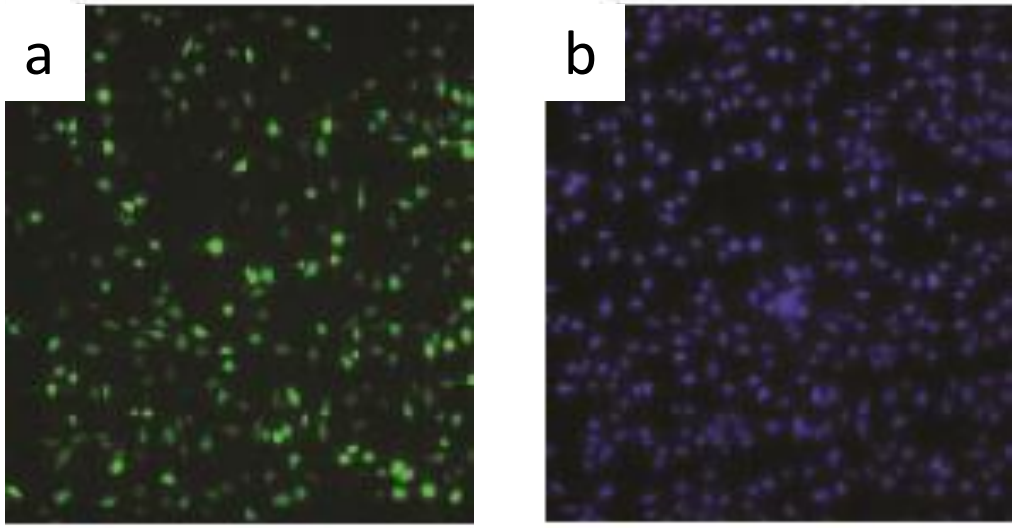
Using fluorescence microscopy to image poration and viability



Porated
(calcein green)

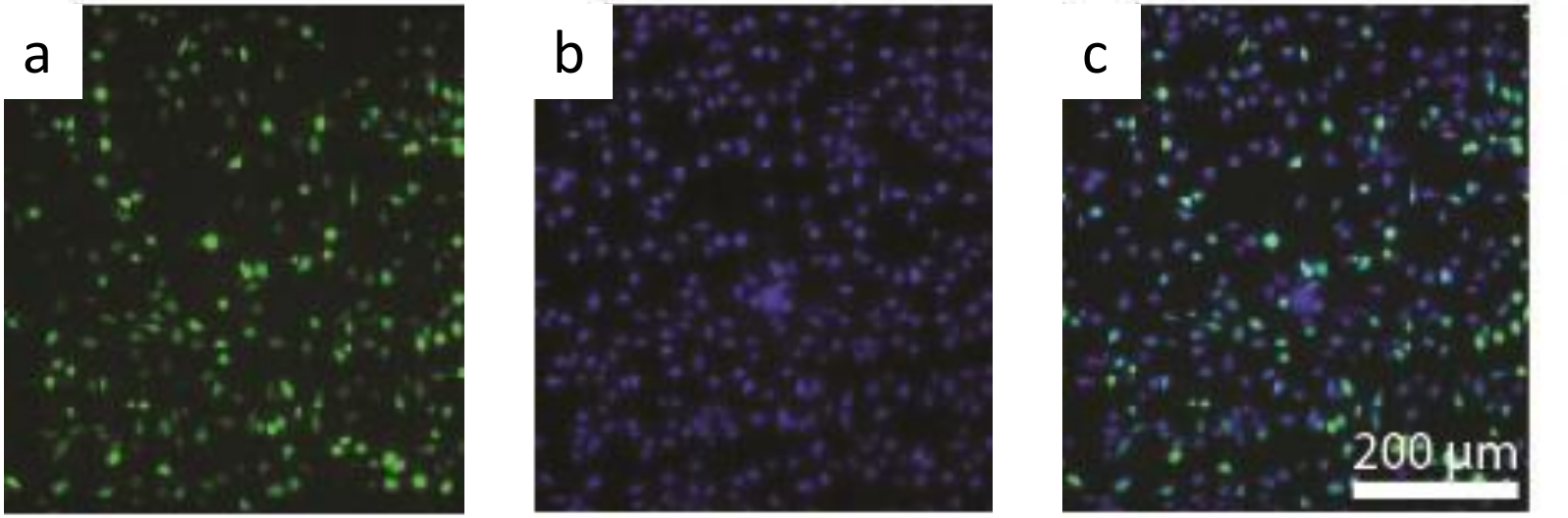
Using fluorescence microscopy to image poration and viability



Porated
(calcein green)

Viable
(calcein AM)

Using fluorescence microscopy to image poration and viability

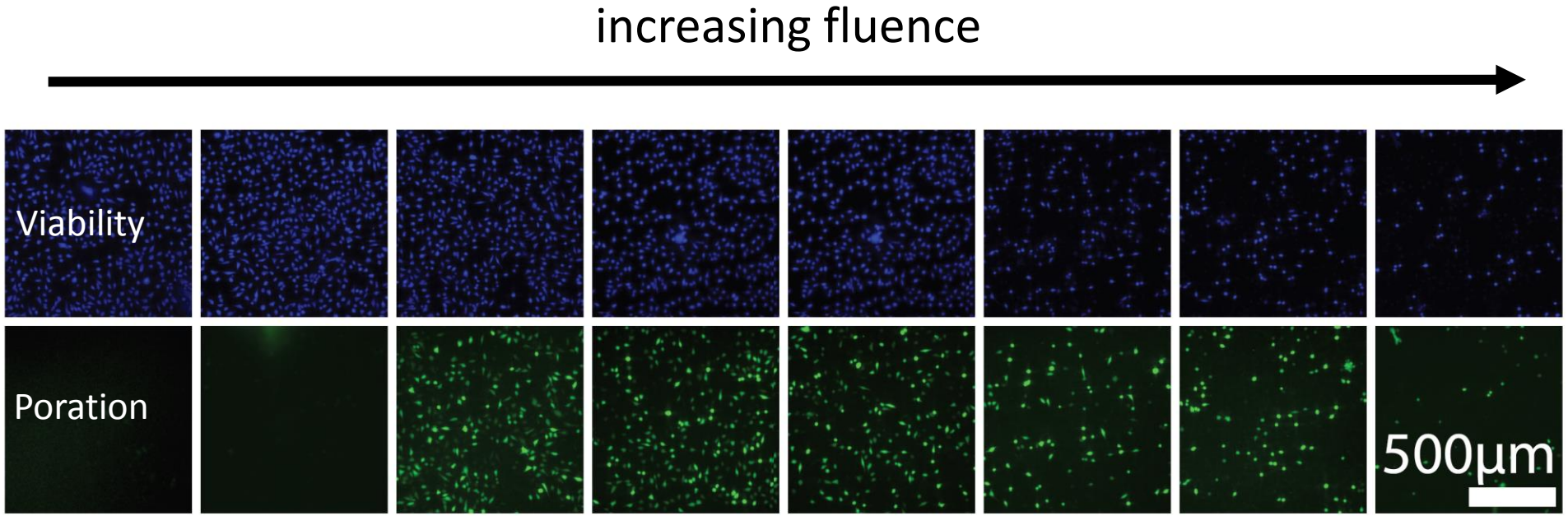


Porated
(calcein green)

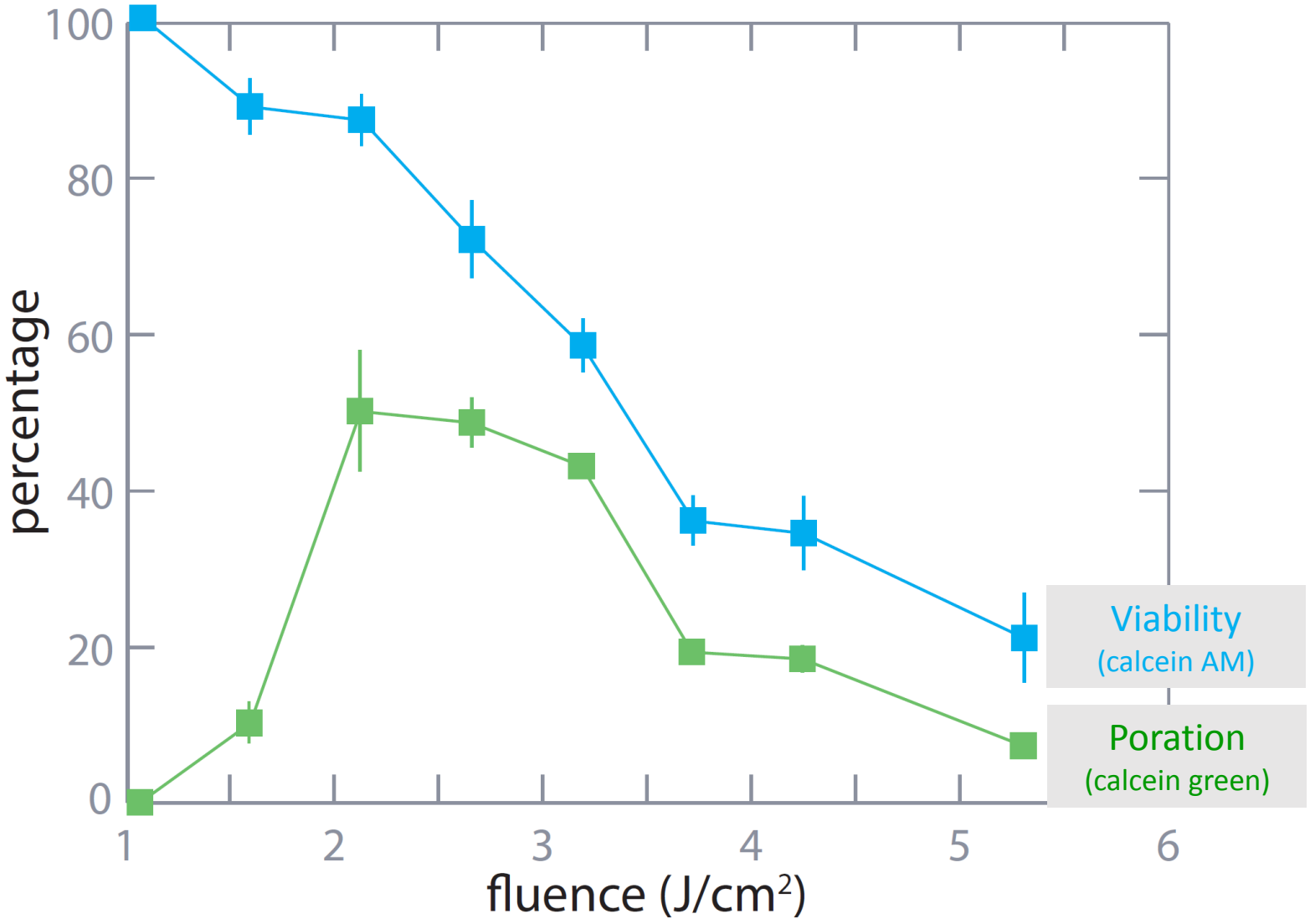
Viable
(calcein AM)

Porated + Viable

Performing cell counting to quantify poration and viability

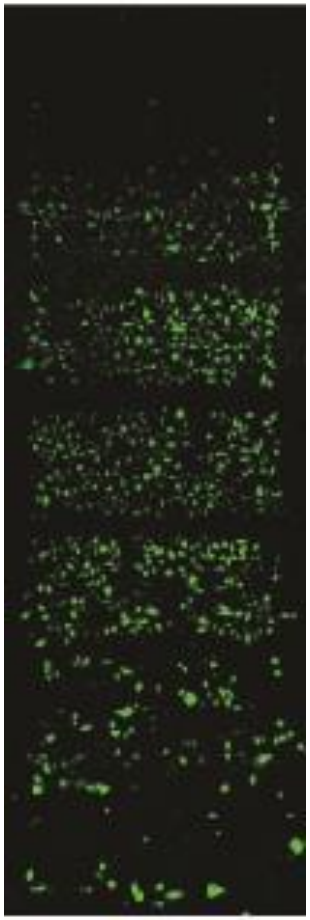


50% of HeLa cells porated with a 40x objective



Poration with a 4x objective

Increasing fluence

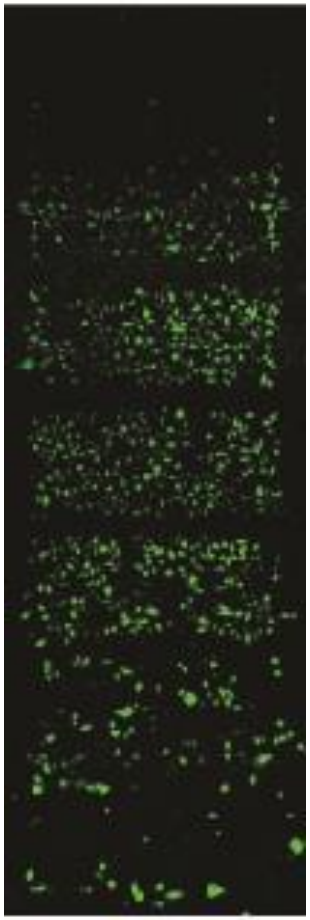


Porated
(calcein green)

Allows poration of specific areas on surface

Poration with a 4x objective

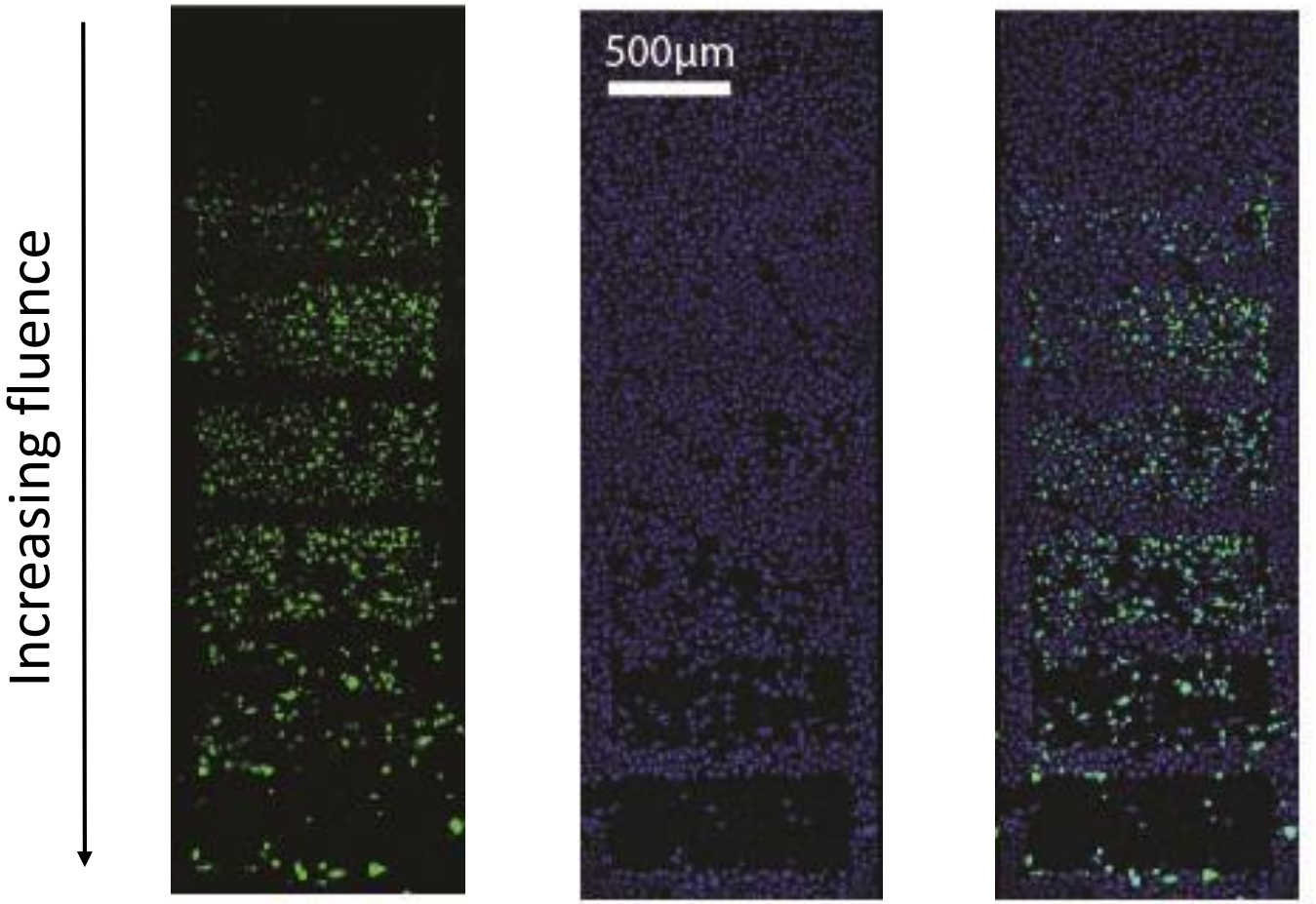
Increasing fluence



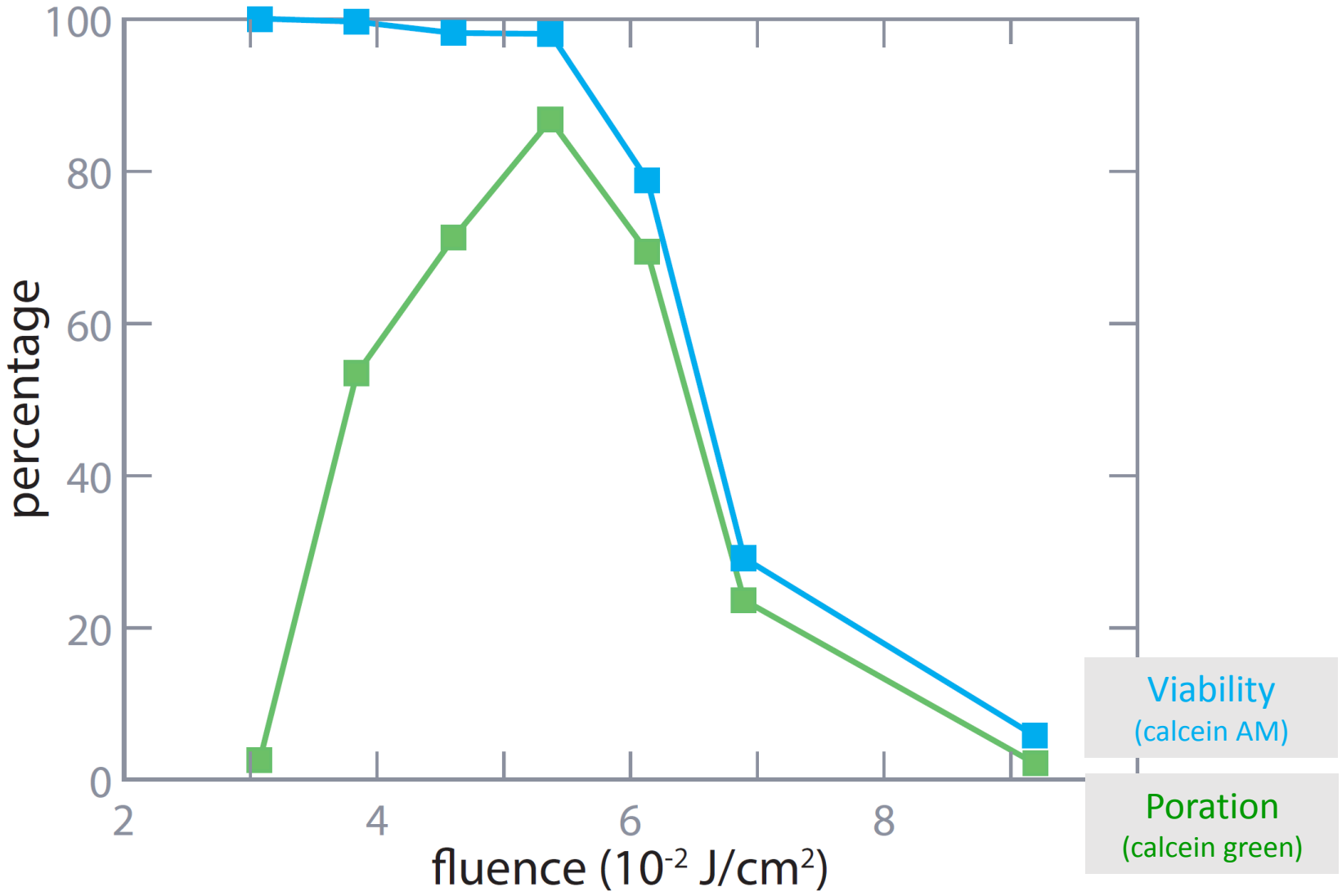
Porated
(calcein green)

Viable
(calcein AM)

Poration with a 4x objective



85% of HeLa cells porated with a 4x objective



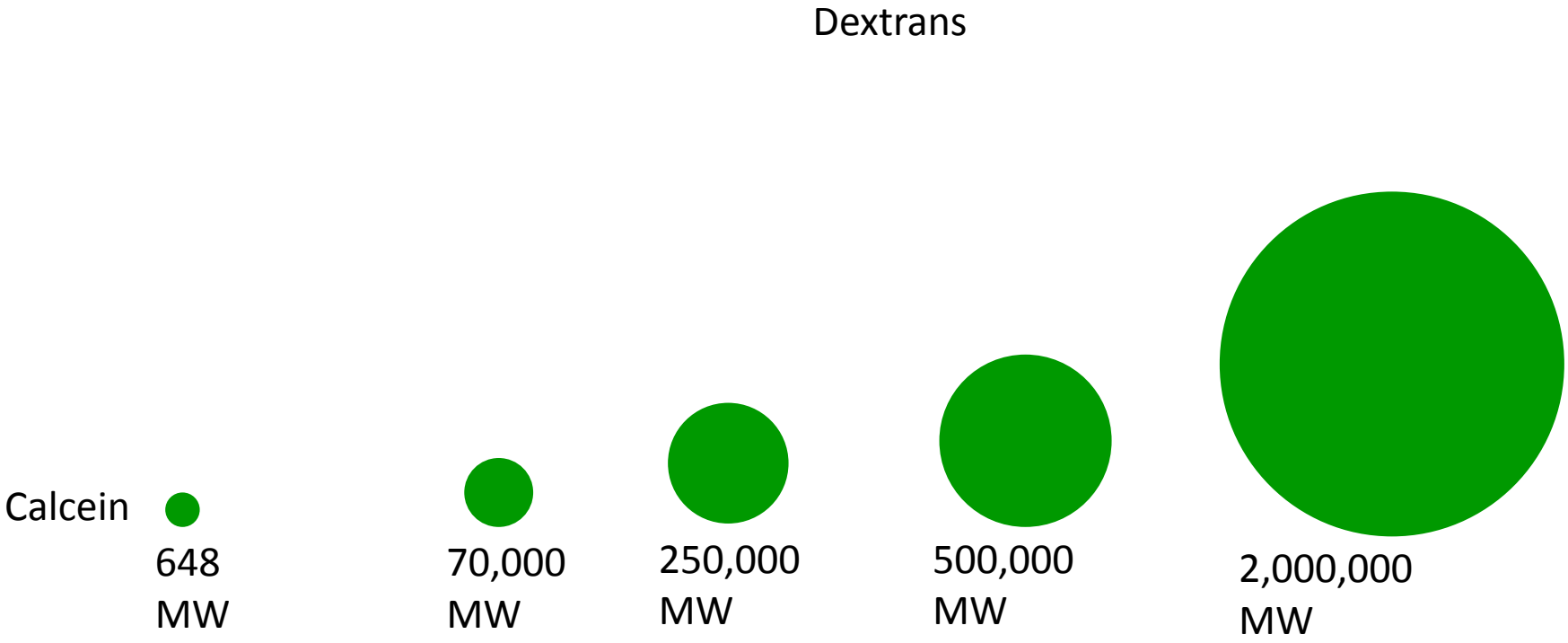
Experiments in progress:
using different dyes to determine pore size

Calcein 

648

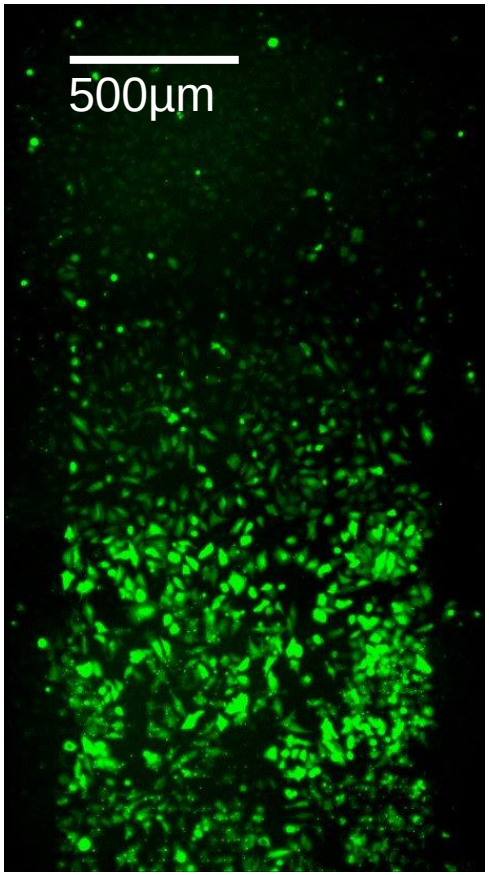
MW

Experiments in progress:
using different dyes to determine pore size



Poration with larger dyes

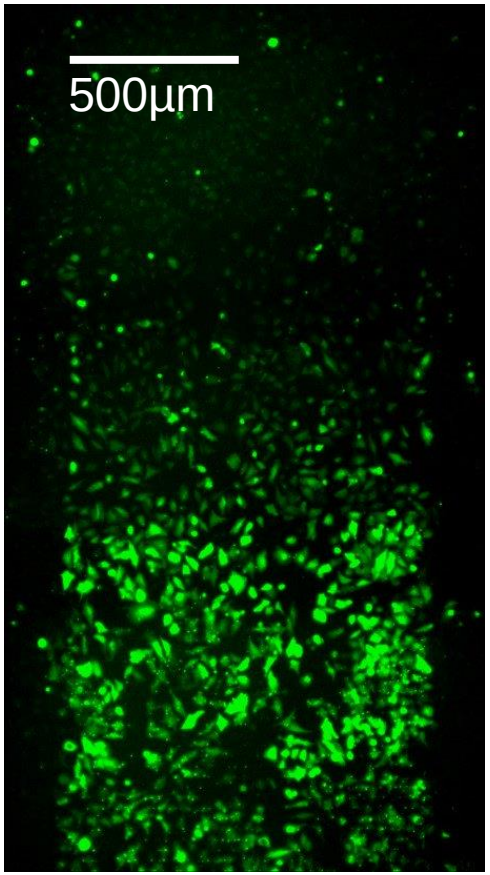
Increasing fluence



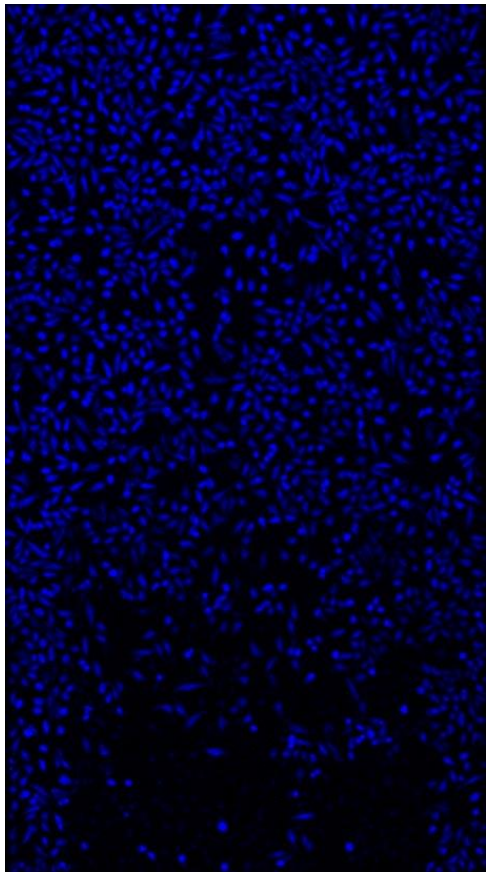
Porated
(dextran 70,000 MW)

Poration with larger dyes

Increasing fluence

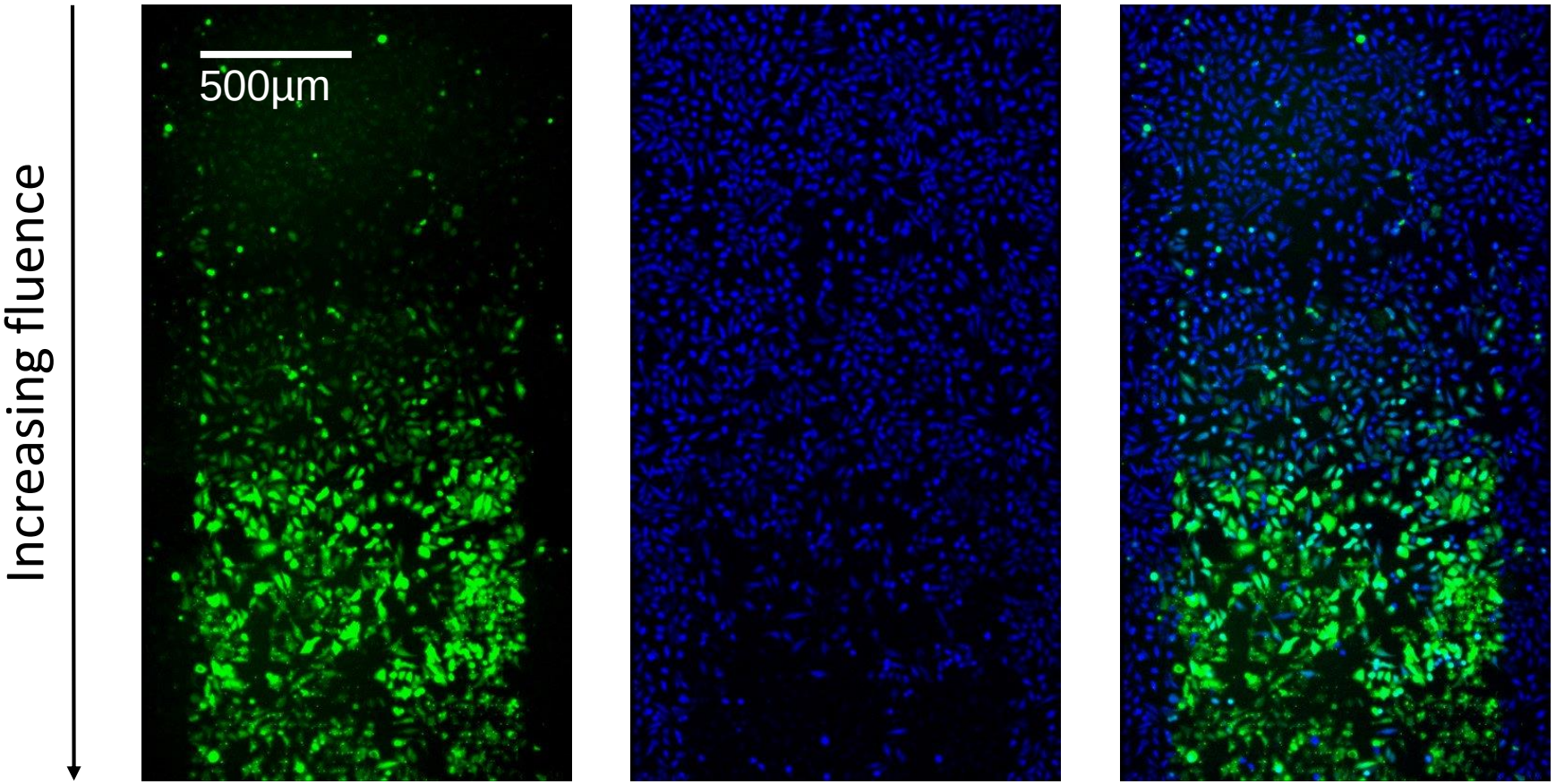


Porated
(dextran 70,000 MW)



Viable
(calcein AM)

Not all dextran-porated cells are viable



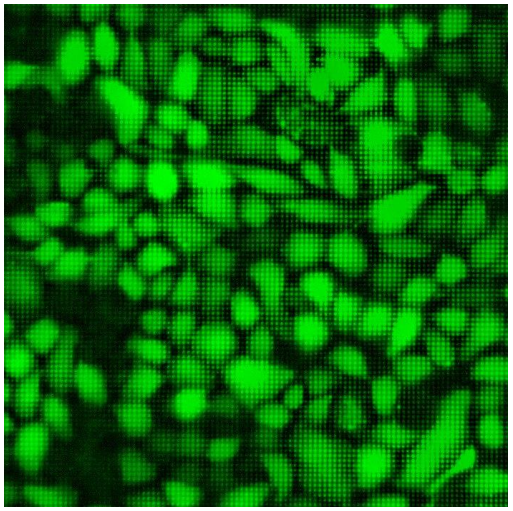
Porated
(dextran 70,000 MW)

Viable
(calcein AM)

Porated + Viable

Experiments in progress: using different cell lines

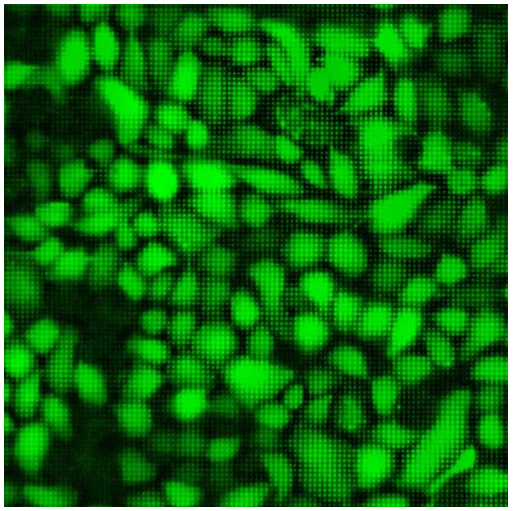
Cell type: MCF10A (human epithelial)



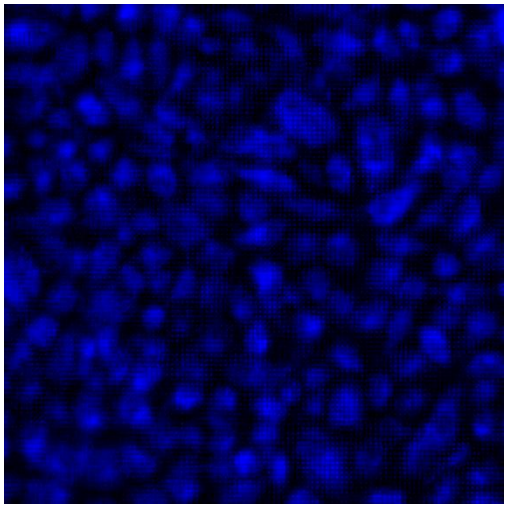
Porated
(calcein green)

Experiments in progress: using different cell lines

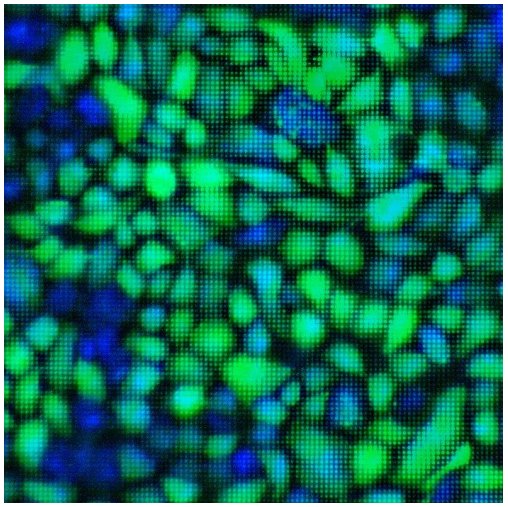
Cell type: MCF10A (human epithelial)



Porated
(calcein green)



Viable
(calcein AM)



Porated + Viable

Experiments in progress: using different cell lines

Cell type: MCF10A (human epithelial)

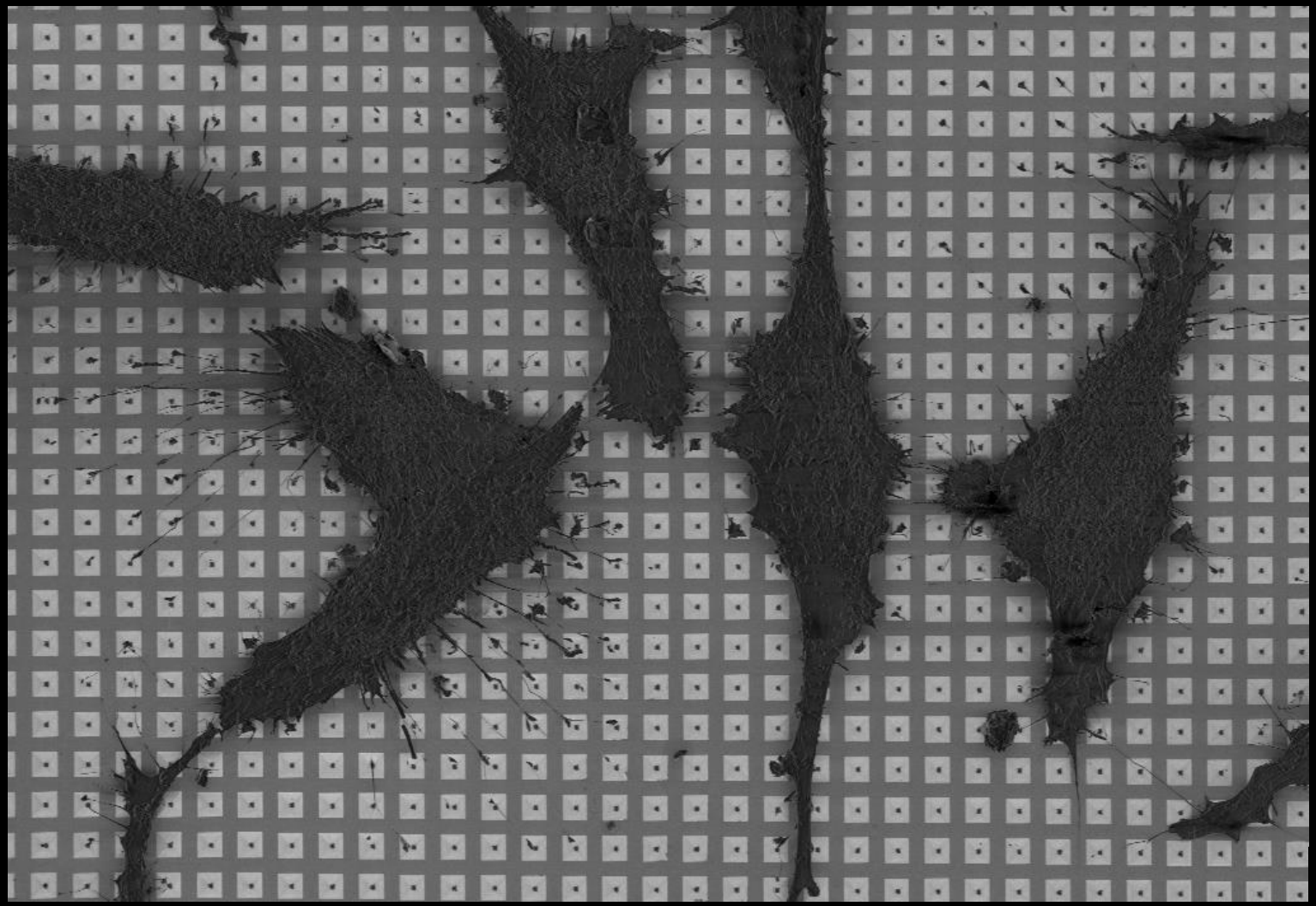
What is happening to the membrane?

Porated
(calcein green)

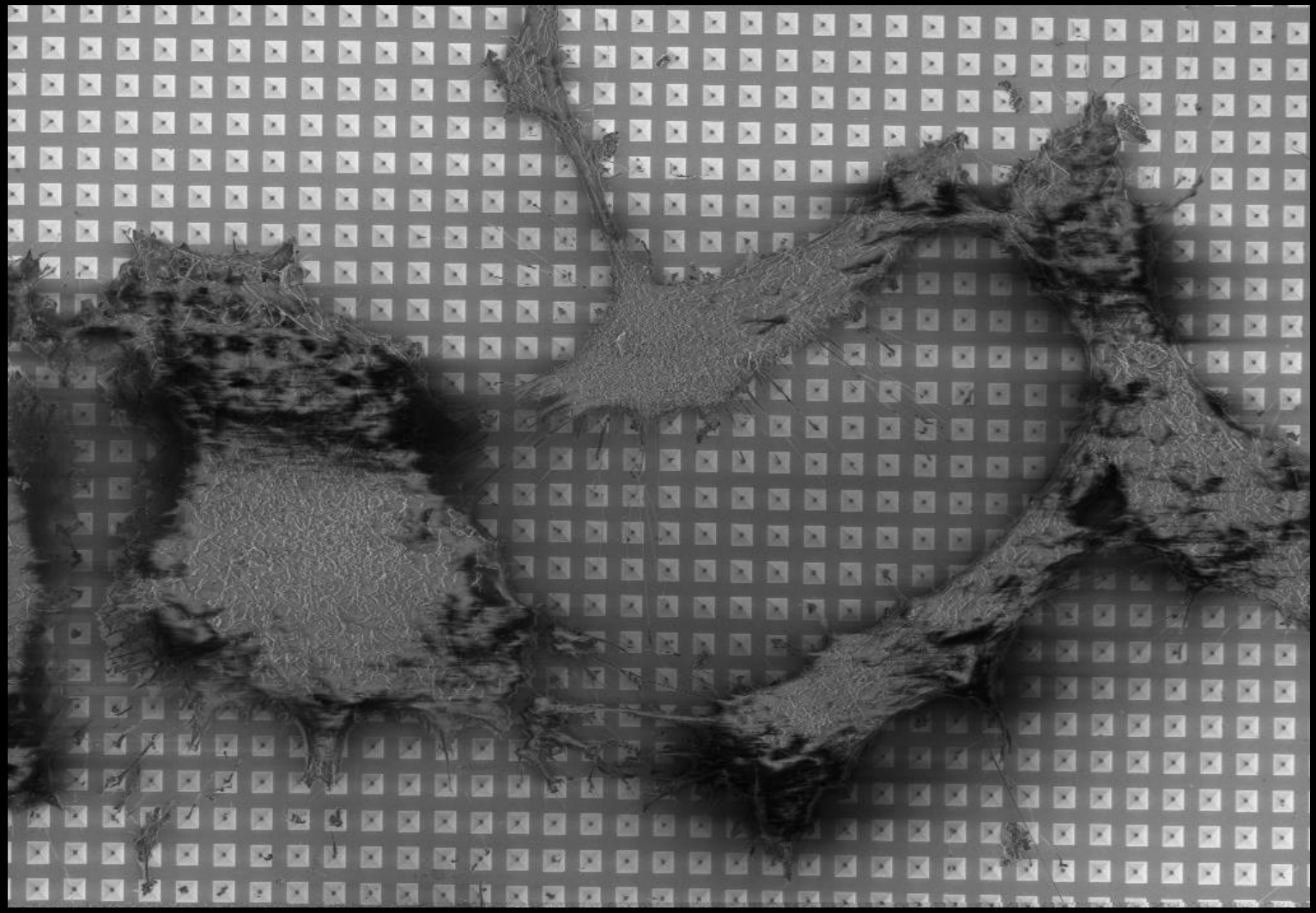
Viable
(calcein AM)

Porated + Viable

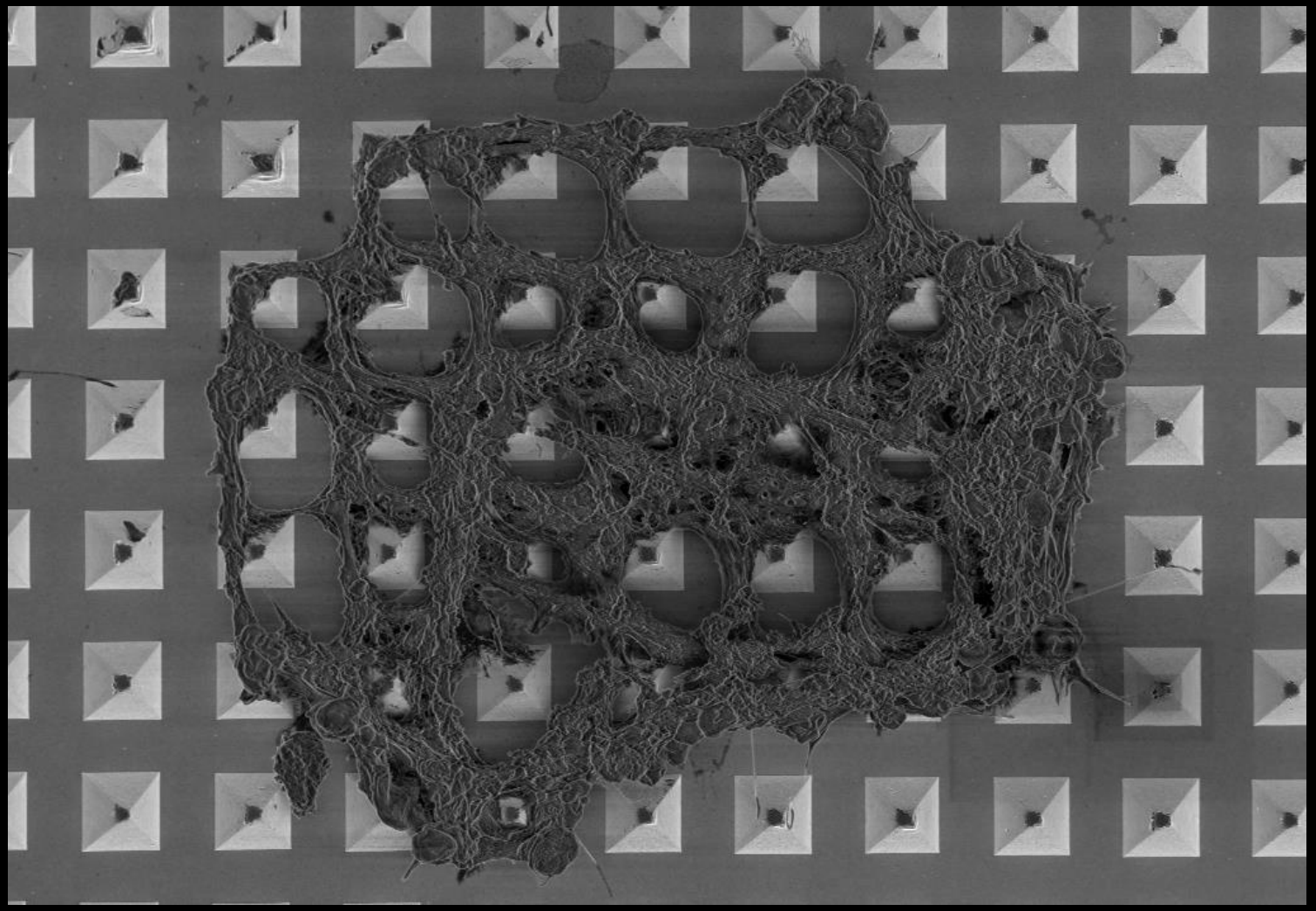
Membrane-substrate interactions determine poration success



Cells uptake dye molecules through pores in membrane

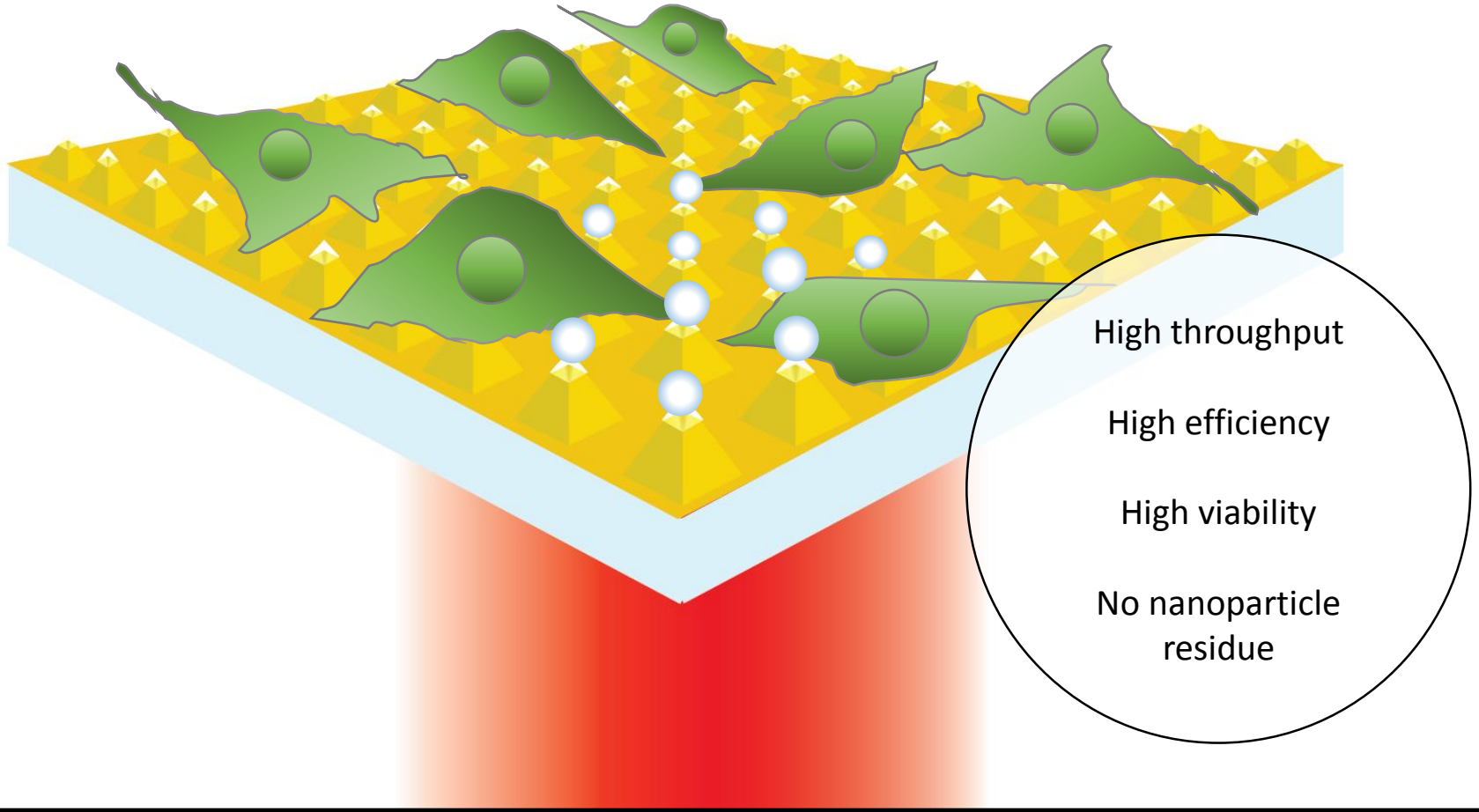


Large pores reduce cell viability

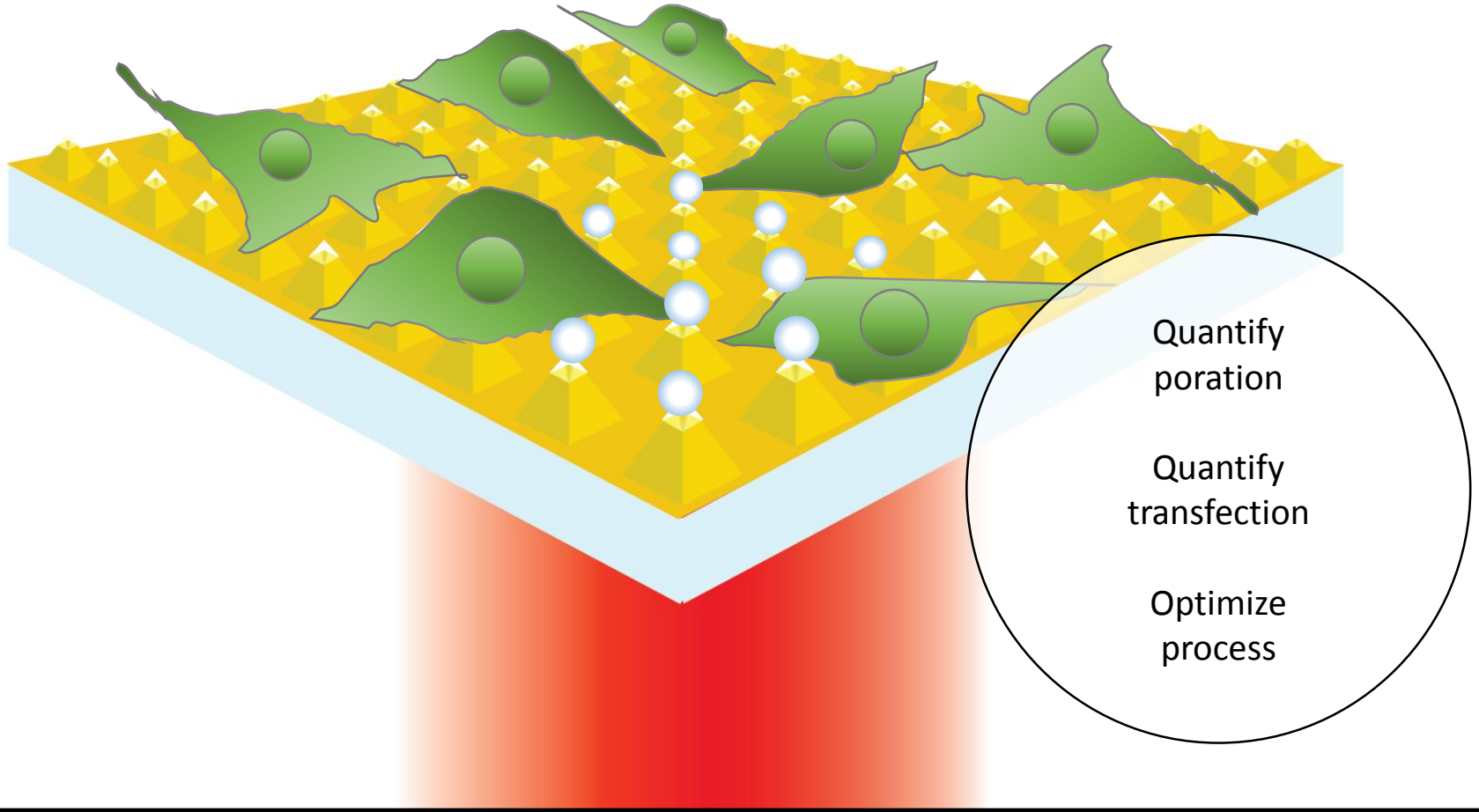


Outlook

Conclusion: Plasmonic substrates for poration



Conclusion: Plasmonic substrates for poration

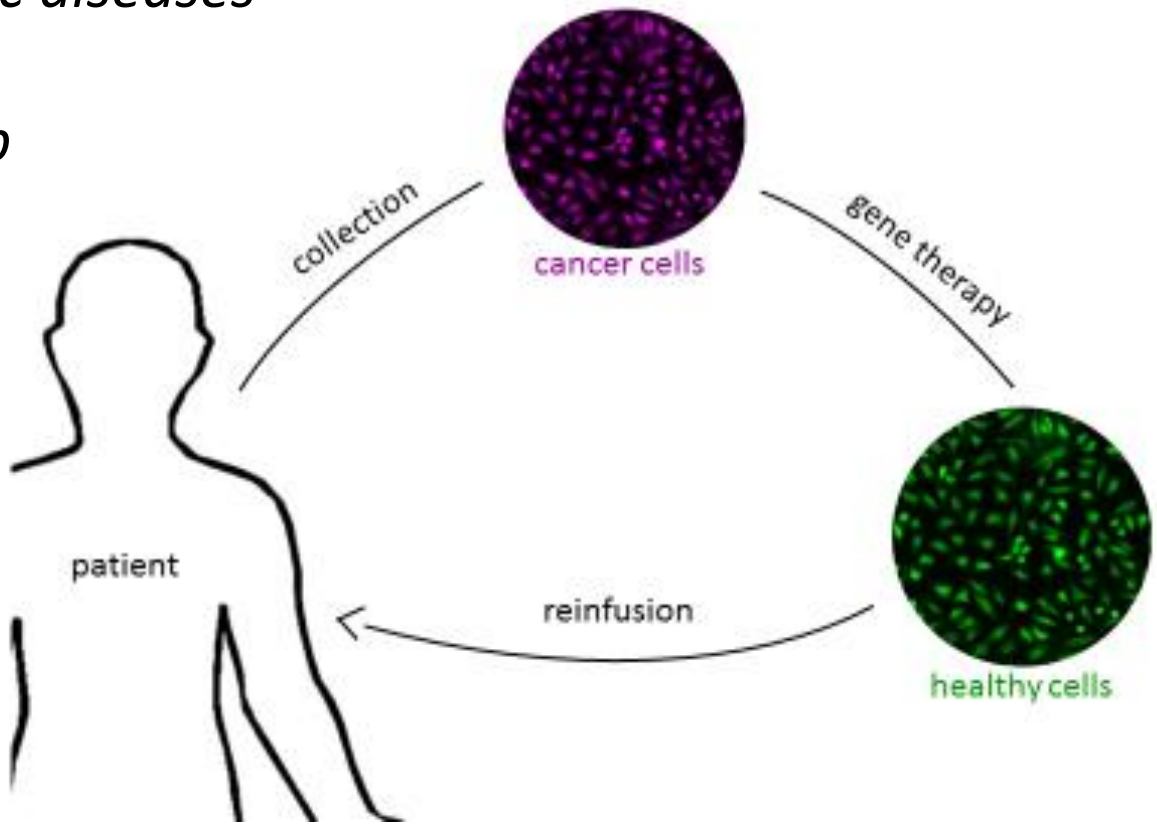


Outlook

Going towards disease-focused applications

Target specific diseases

Lab-on-a-chip



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Talk later in this session:

Design, simulation, and fabrication of plasmonic pyramid substrate for cell transfection (Paper 9355-16)- Marinus Huber
Time: 5:40 PM - 6:00 PM

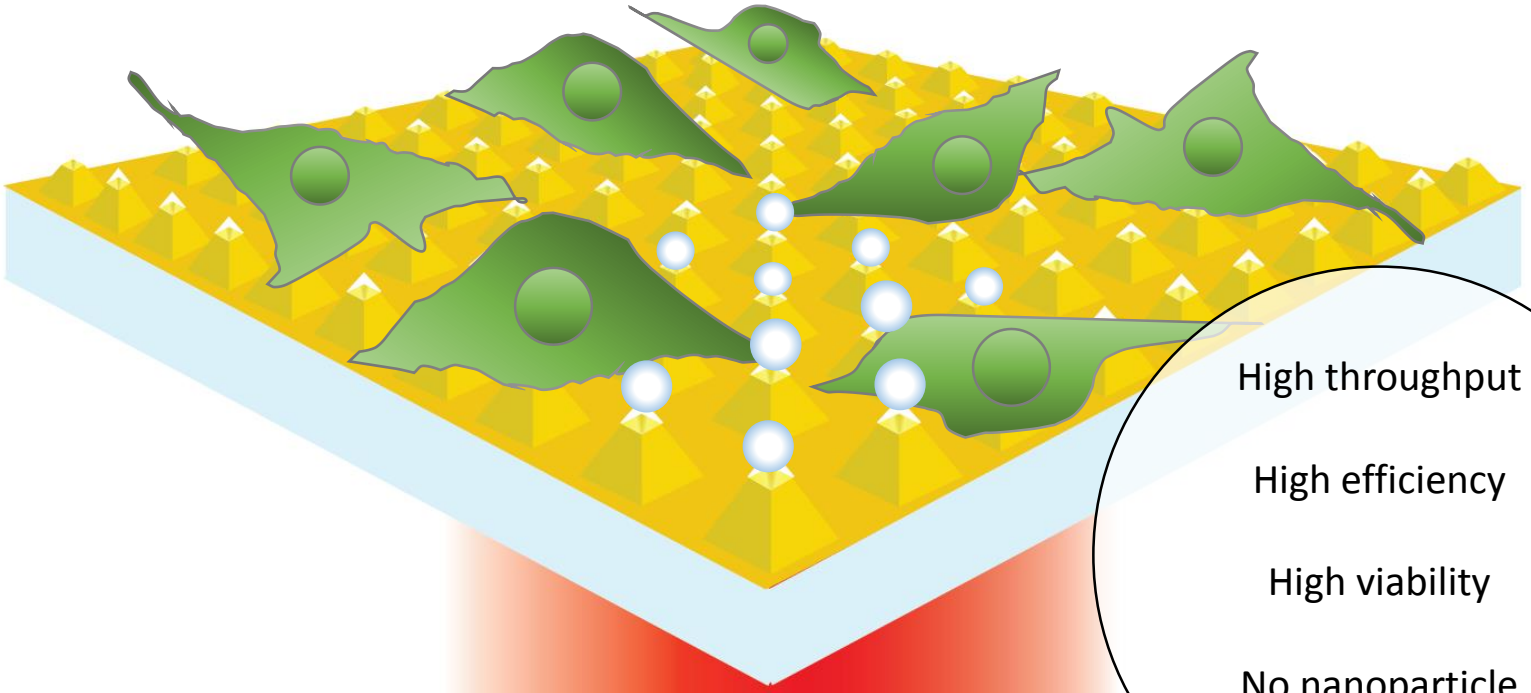
Tuesday poster session:

Plasmonic substrates for cell transfection (Paper 9355-48)- Marinna Madrid
Time: 6:00 PM - 8:00 PM

Thank you for your attention!

saklayen@physics.harvard.edu mazur.harvard.edu

Plasmonic substrates for poration



High throughput

High efficiency

High viability

No nanoparticle residue

Thank you for your attention!

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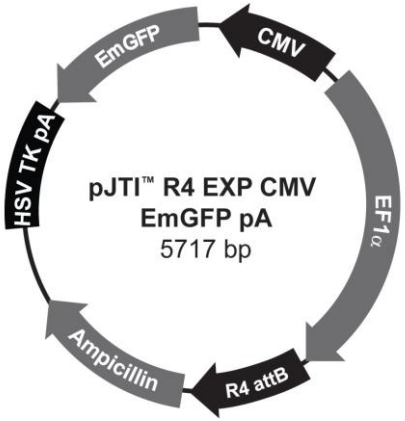
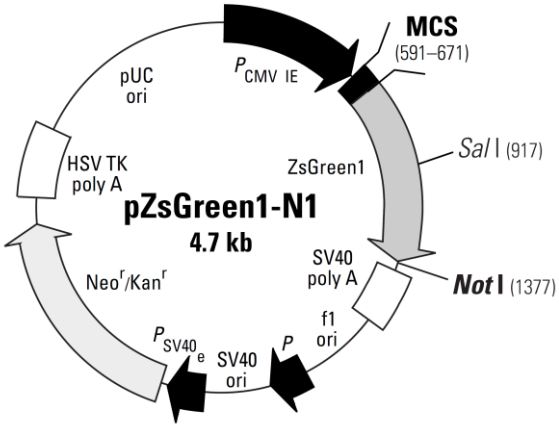
Extra slides

Experiments in progress: transfection with DNA plasmids

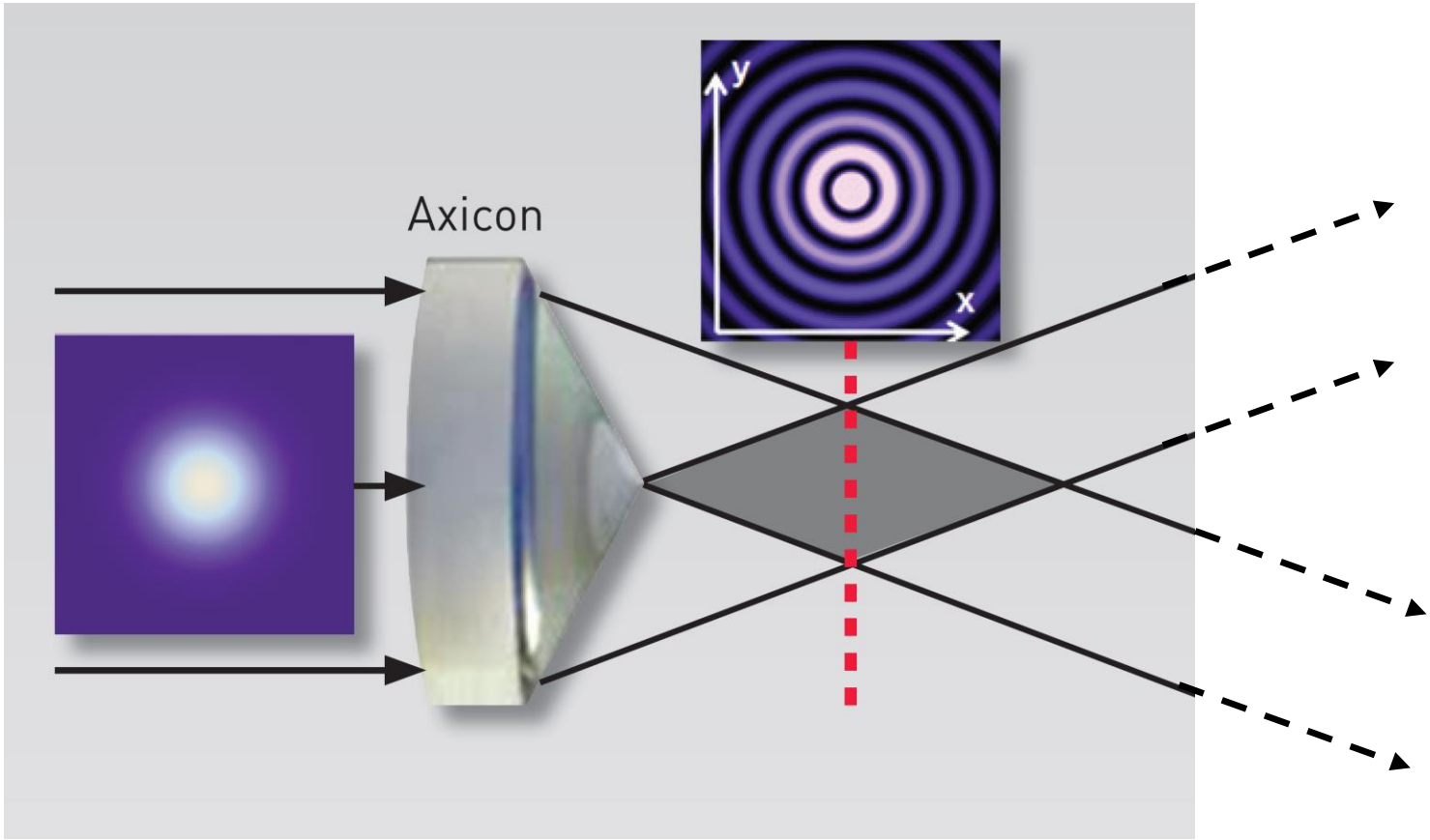


Transient
GFP expression



Permanent
GFP expression

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.

Making the leap