

Shining light on cells to cure diseases



SPIE LASE
Photonics West 2017
San Francisco, CA, 29 January 2017





2 AM TO 6 AM
EVERYDAY
INCLUDING HOLIDAYS
STREET SWEEPING*

Freemans

SAKS FIFTH AVE

HOTEL
STRATFORD

ITSH



Nabiha Saklayen



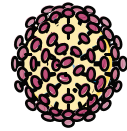
Marinna Madrid



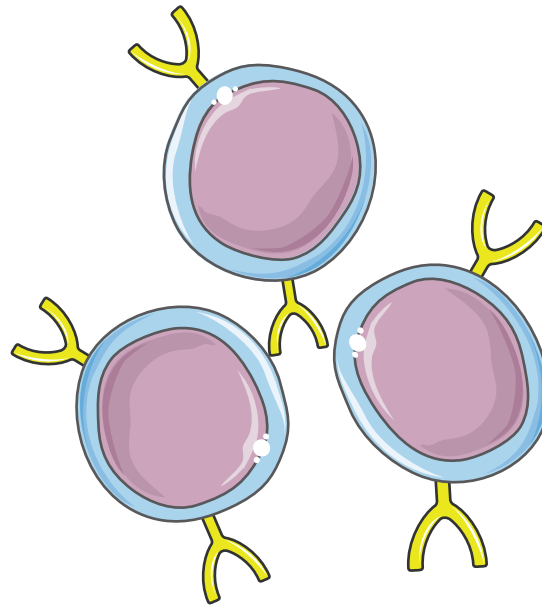
Eric Mazur



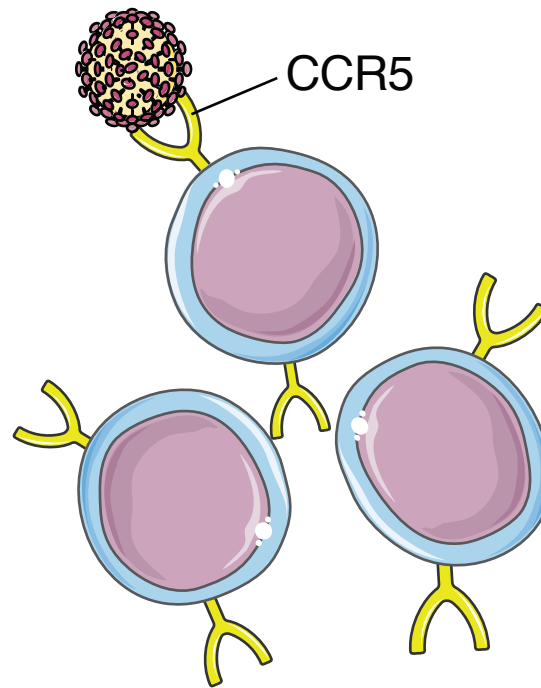
eric_mazur



HIV

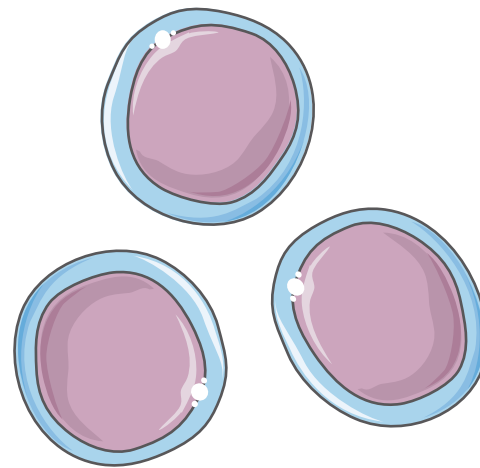


white blood cells



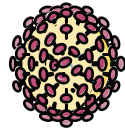
white blood cells

Mandal *et al.*, *Cell Stem Cell* 15, 643 (2014)

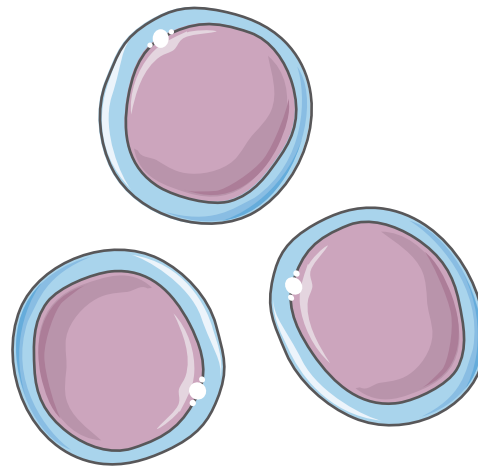


white blood cells

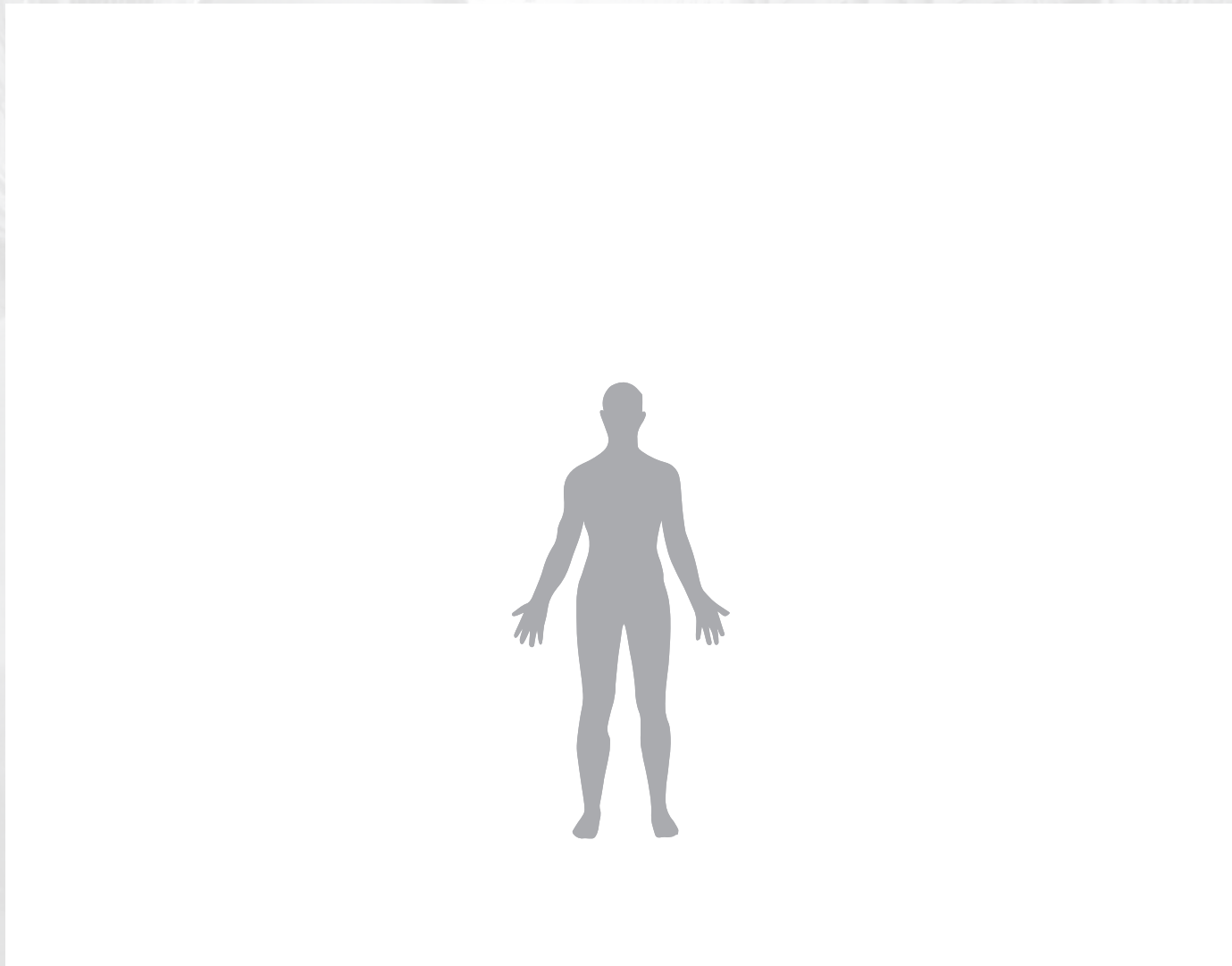
Mandal *et al.*, *Cell Stem Cell* 15, 643 (2014)



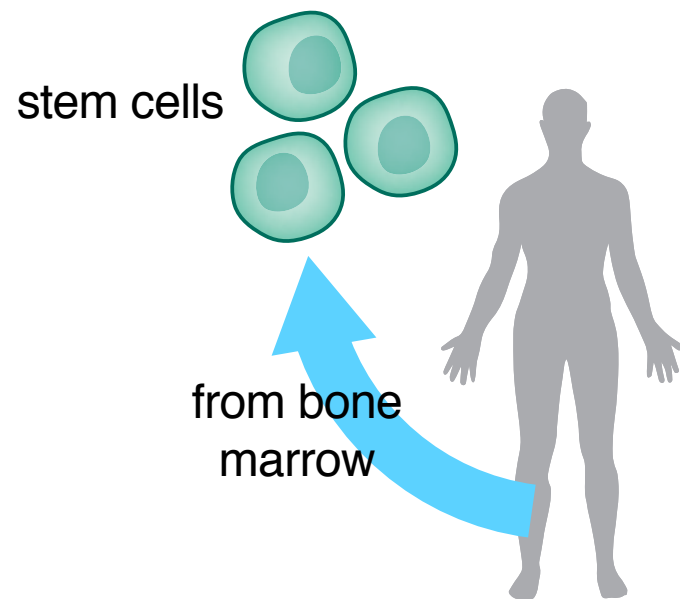
HIV



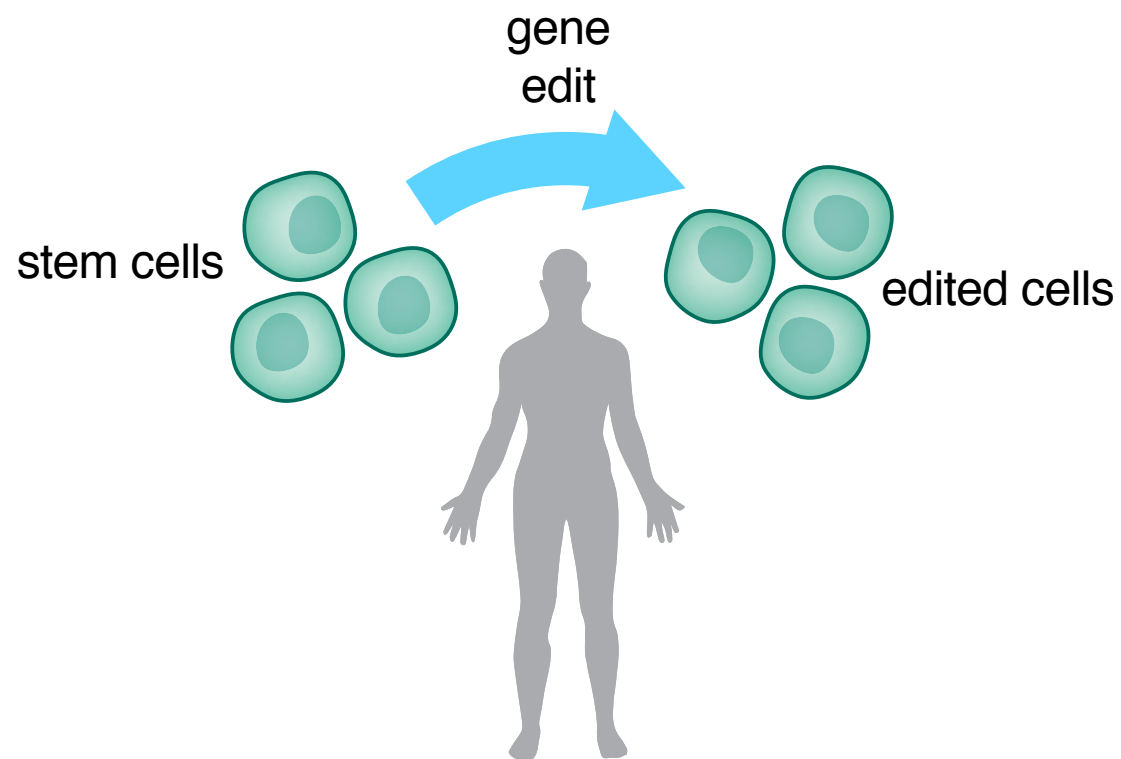
white blood cells



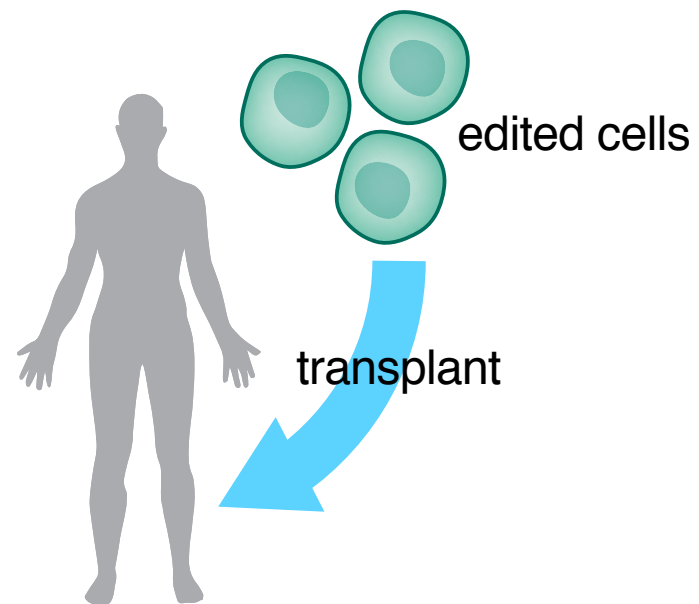
Mandal *et al.*, *Cell Stem Cell* 15, 643 (2014)



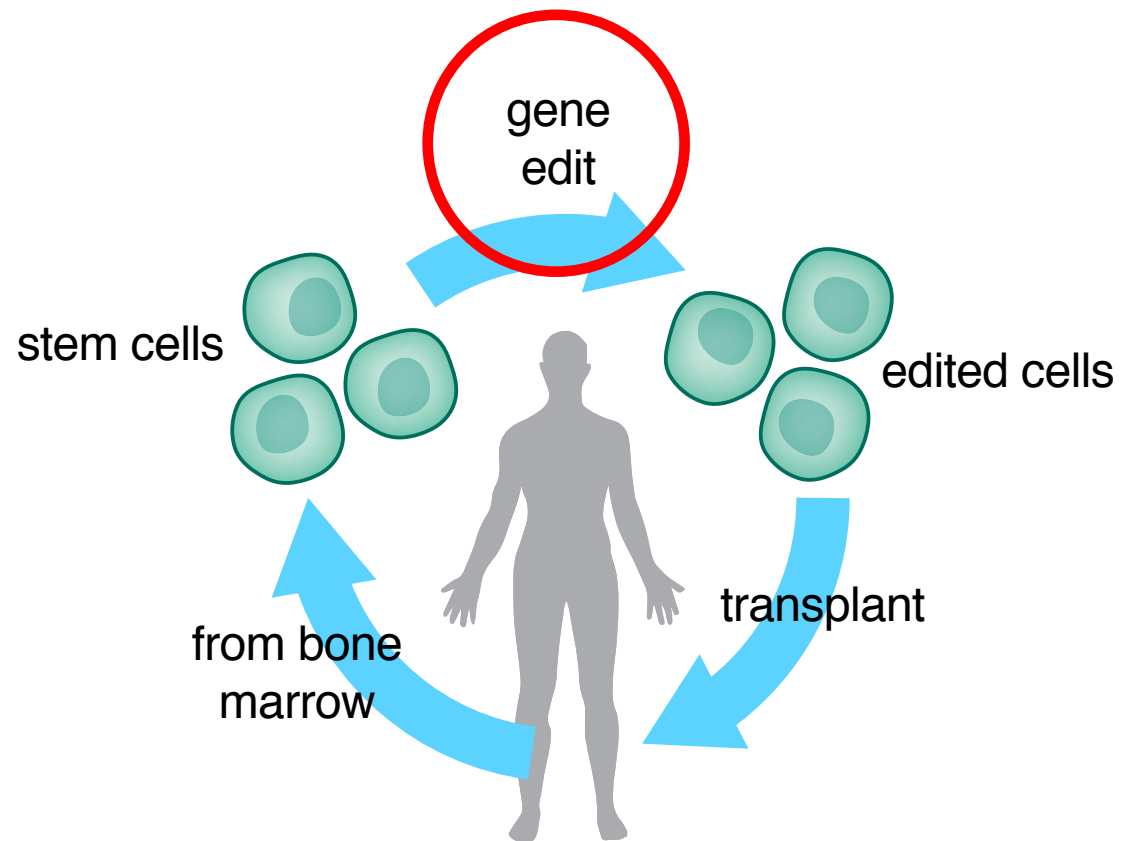
Mandal *et al.*, *Cell Stem Cell* 15, 643 (2014)



Mandal *et al.*, *Cell Stem Cell* 15, 643 (2014)



Mandal *et al.*, *Cell Stem Cell* 15, 643 (2014)



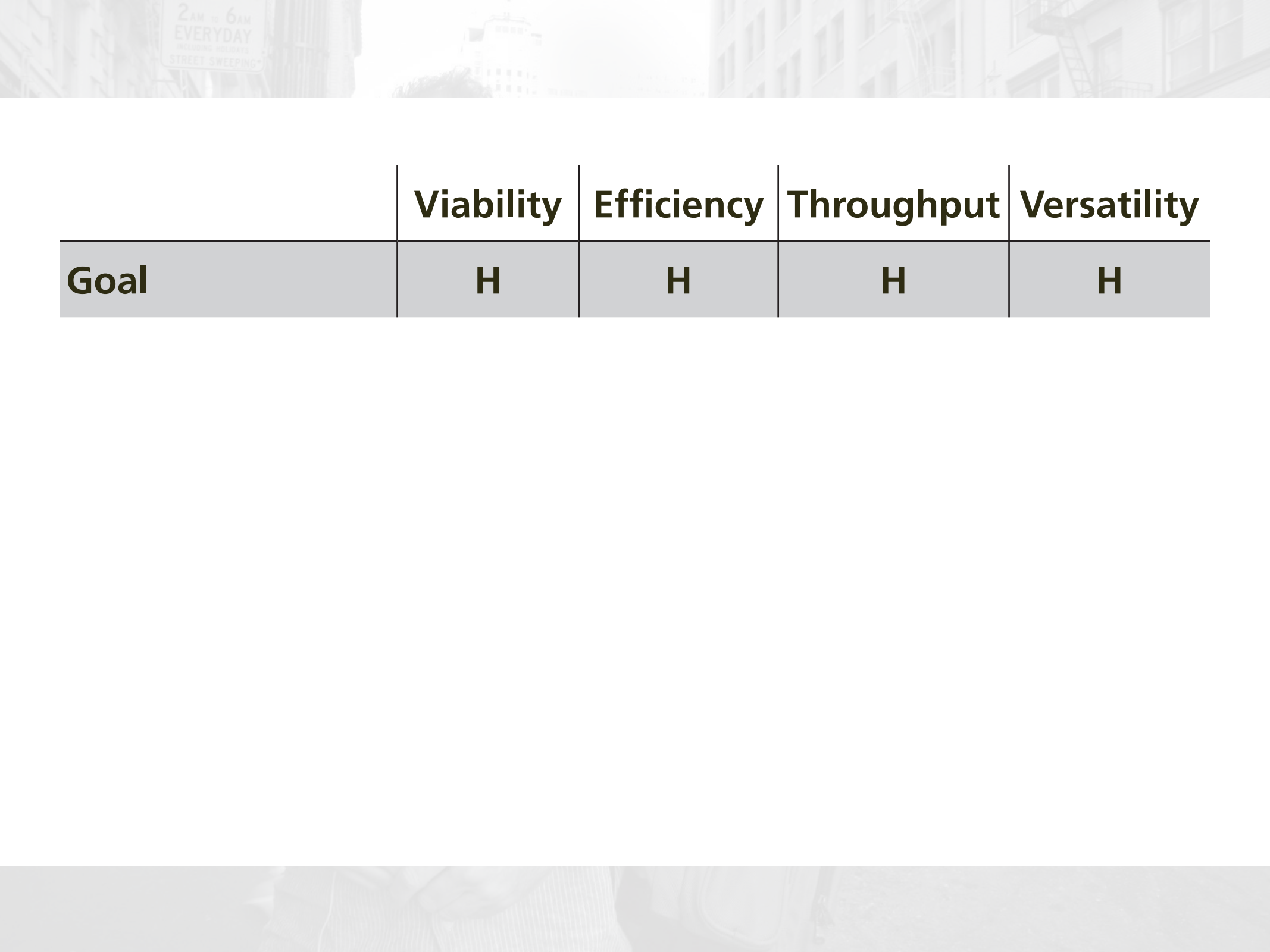
Mandal *et al.*, *Cell Stem Cell* 15, 643 (2014)

A grayscale photograph of a man walking on a city street. He is smiling, wearing a striped button-down shirt, a backpack, and white earbuds. He is looking towards the camera. The background shows a busy city street with buildings, a tram, and various signs. A sign for 'HOTEL STRATFORD' is visible on the right, and a sign for 'SAKS FIFTH AVENUE' is visible in the background. A sign for '2AM to 6AM EVERYDAY INCLUDING HOLIDAYS STREET SWEEPING' is visible on the left. A sign for 'TISH' is visible on the tram. The text 'How can we efficiently deliver cargo to cells?' is overlaid in the center of the image.

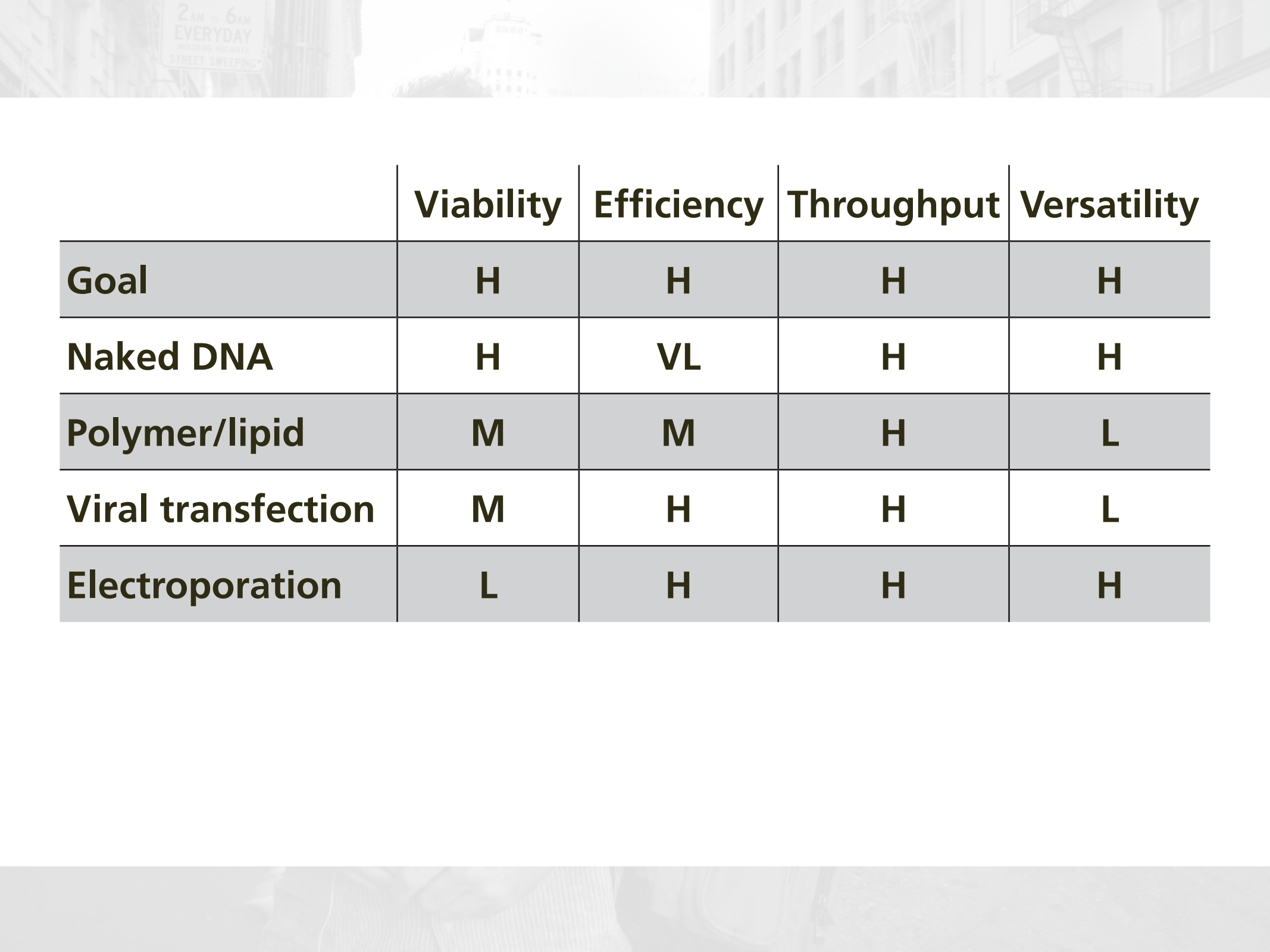
How can we efficiently deliver cargo to cells?

A grayscale photograph of a man walking on a city street. He is smiling, wearing a striped button-down shirt, a backpack, and white earbuds. The background shows a busy urban environment with tall buildings, a tram, and various street signs. One sign reads "2AM to 6AM EVERYDAY INCLUDING HOLIDAYS STREET SWEEPING*", another says "HOTEL STRATFORD", and a third mentions "SAKS FIFTH AVE".

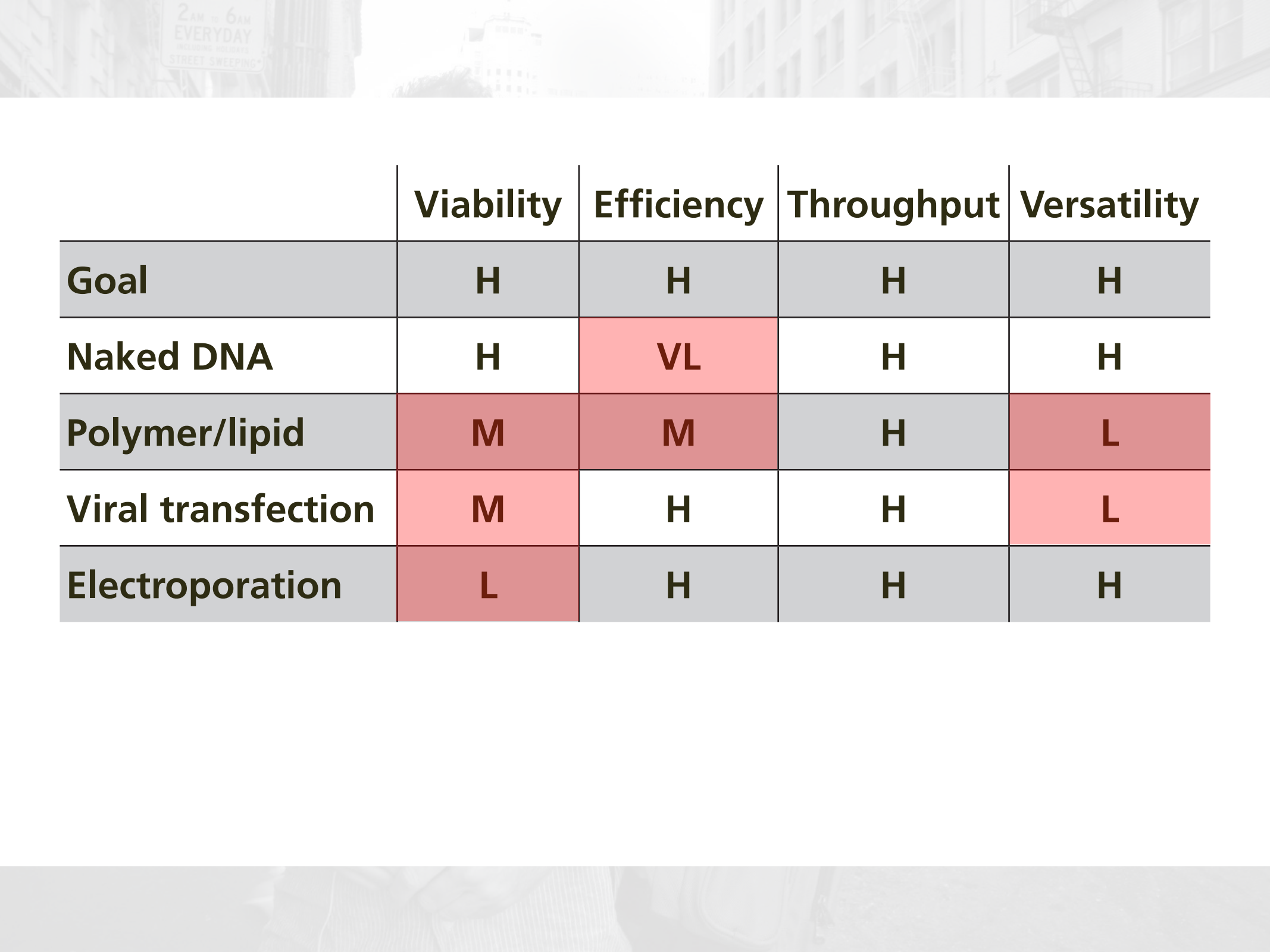
specifically, gene-editing tools to stem cells



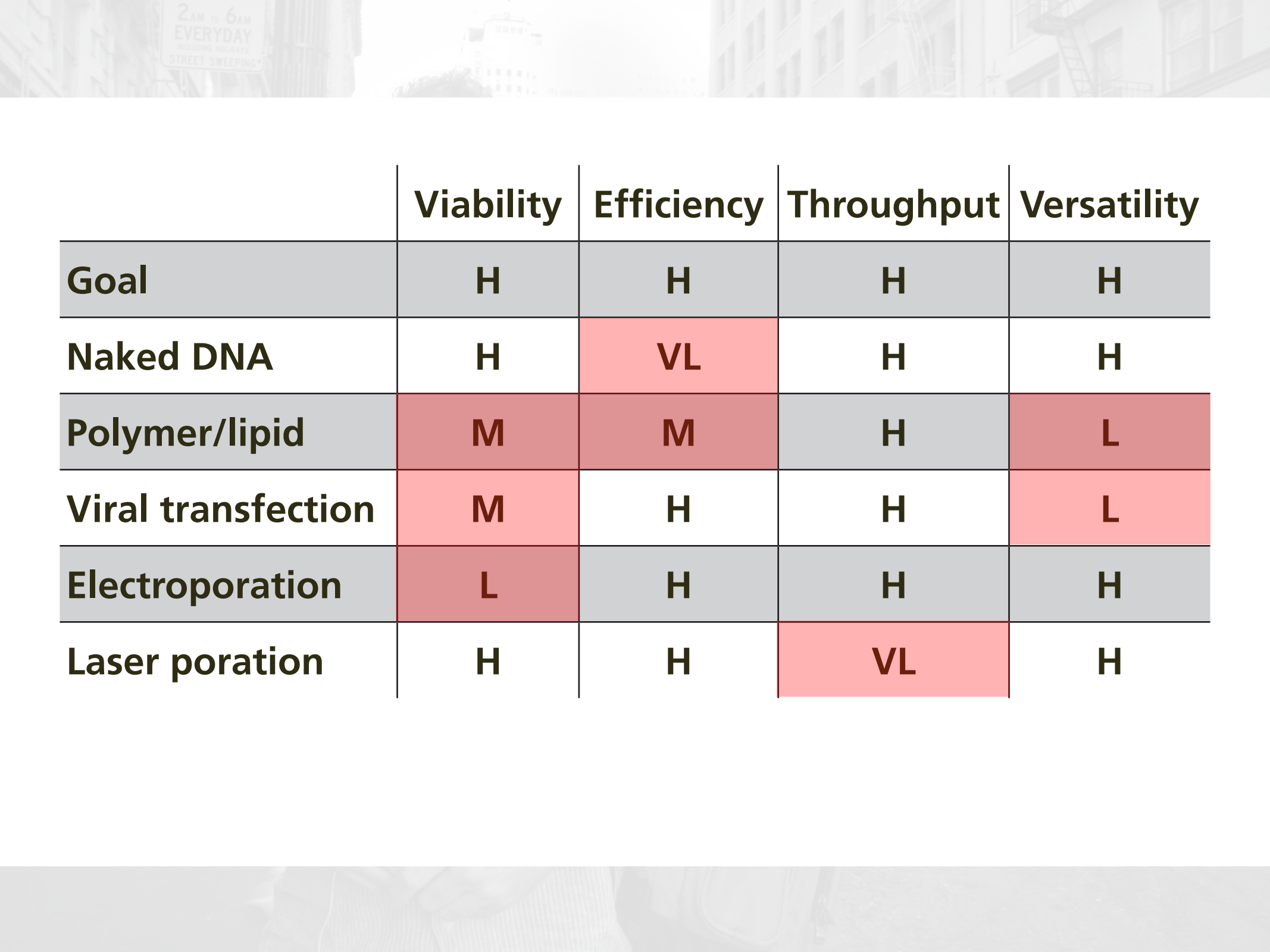
	Viability	Efficiency	Throughput	Versatility
Goal	H	H	H	H



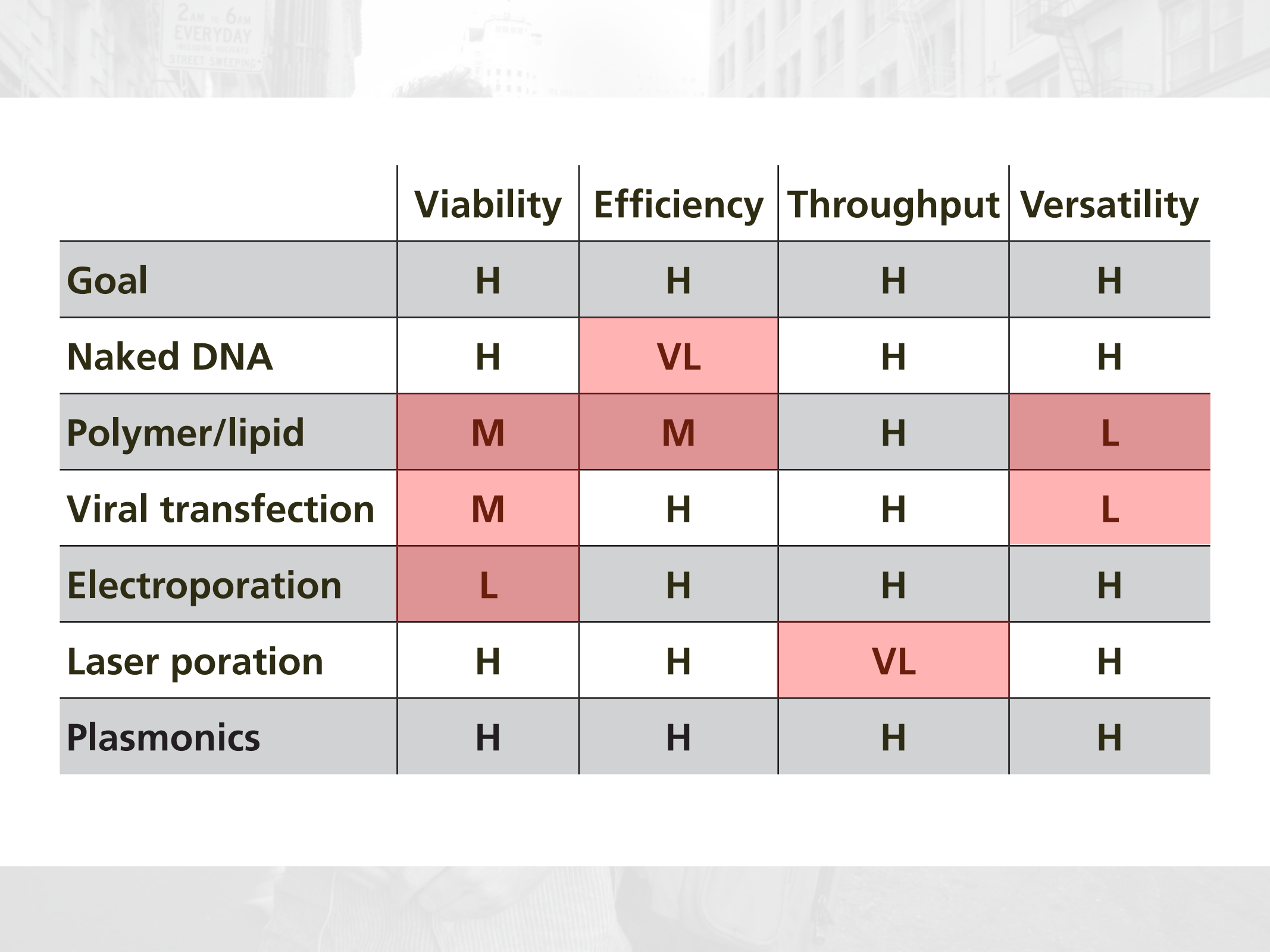
	Viability	Efficiency	Throughput	Versatility
Goal	H	H	H	H
Naked DNA	H	VL	H	H
Polymer/lipid	M	M	H	L
Viral transfection	M	H	H	L
Electroporation	L	H	H	H



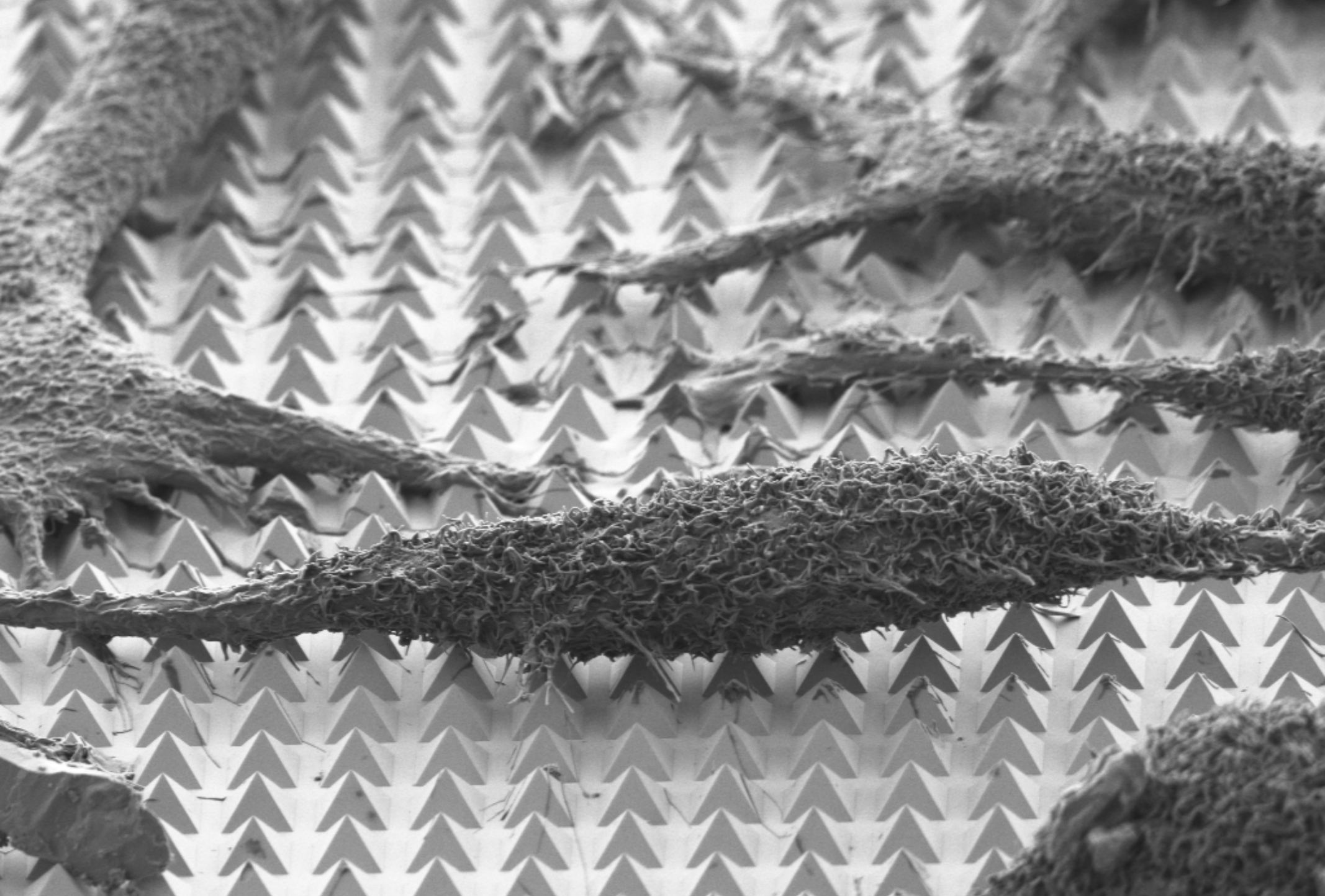
	Viability	Efficiency	Throughput	Versatility
Goal	H	H	H	H
Naked DNA	H	VL	H	H
Polymer/lipid	M	M	H	L
Viral transfection	M	H	H	L
Electroporation	L	H	H	H



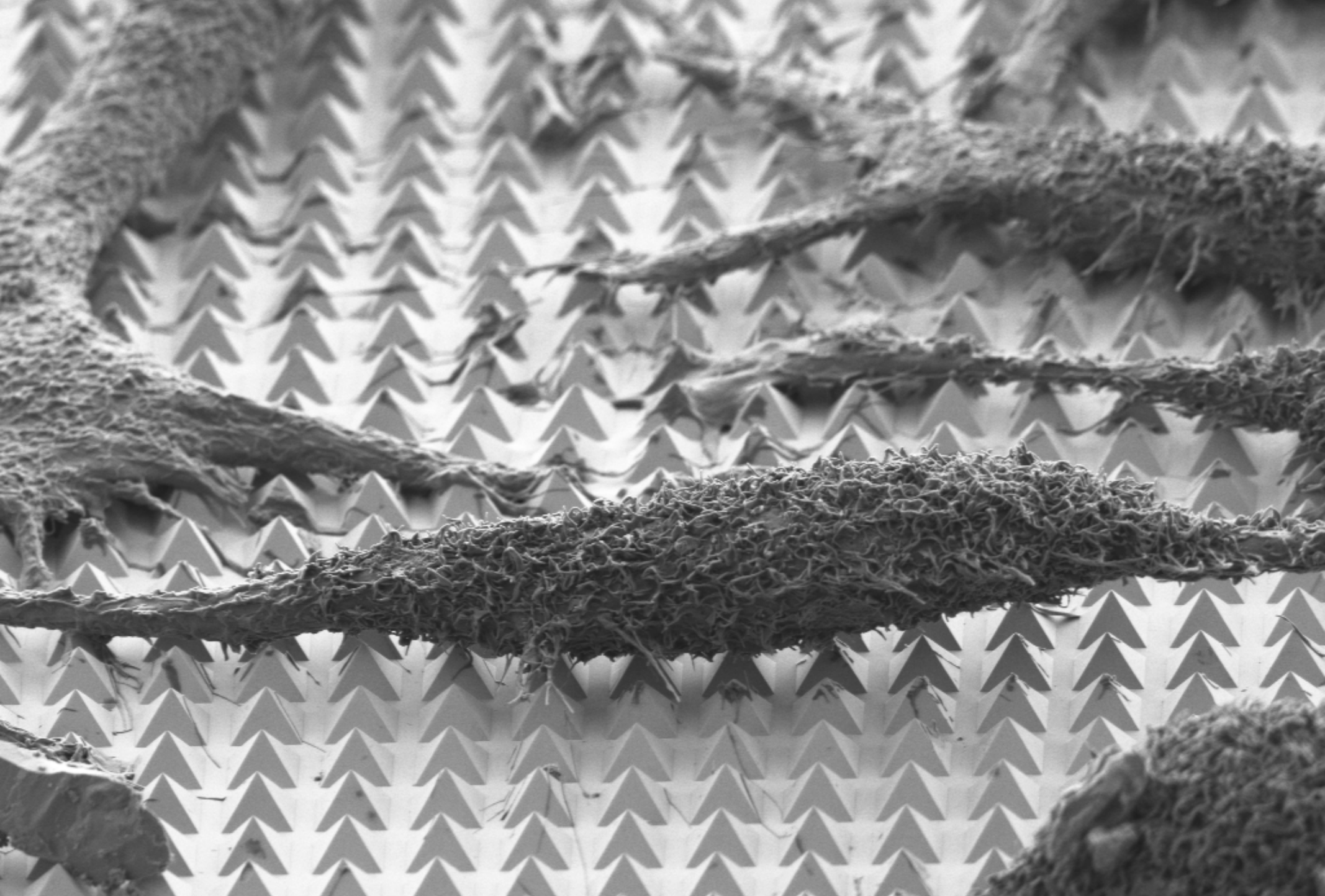
	Viability	Efficiency	Throughput	Versatility
Goal	H	H	H	H
Naked DNA	H	VL	H	H
Polymer/lipid	M	M	H	L
Viral transfection	M	H	H	L
Electroporation	L	H	H	H
Laser poration	H	H	VL	H



	Viability	Efficiency	Throughput	Versatility
Goal	H	H	H	H
Naked DNA	H	VL	H	H
Polymer/lipid	M	M	H	L
Viral transfection	M	H	H	L
Electroporation	L	H	H	H
Laser poration	H	H	VL	H
Plasmonics	H	H	H	H

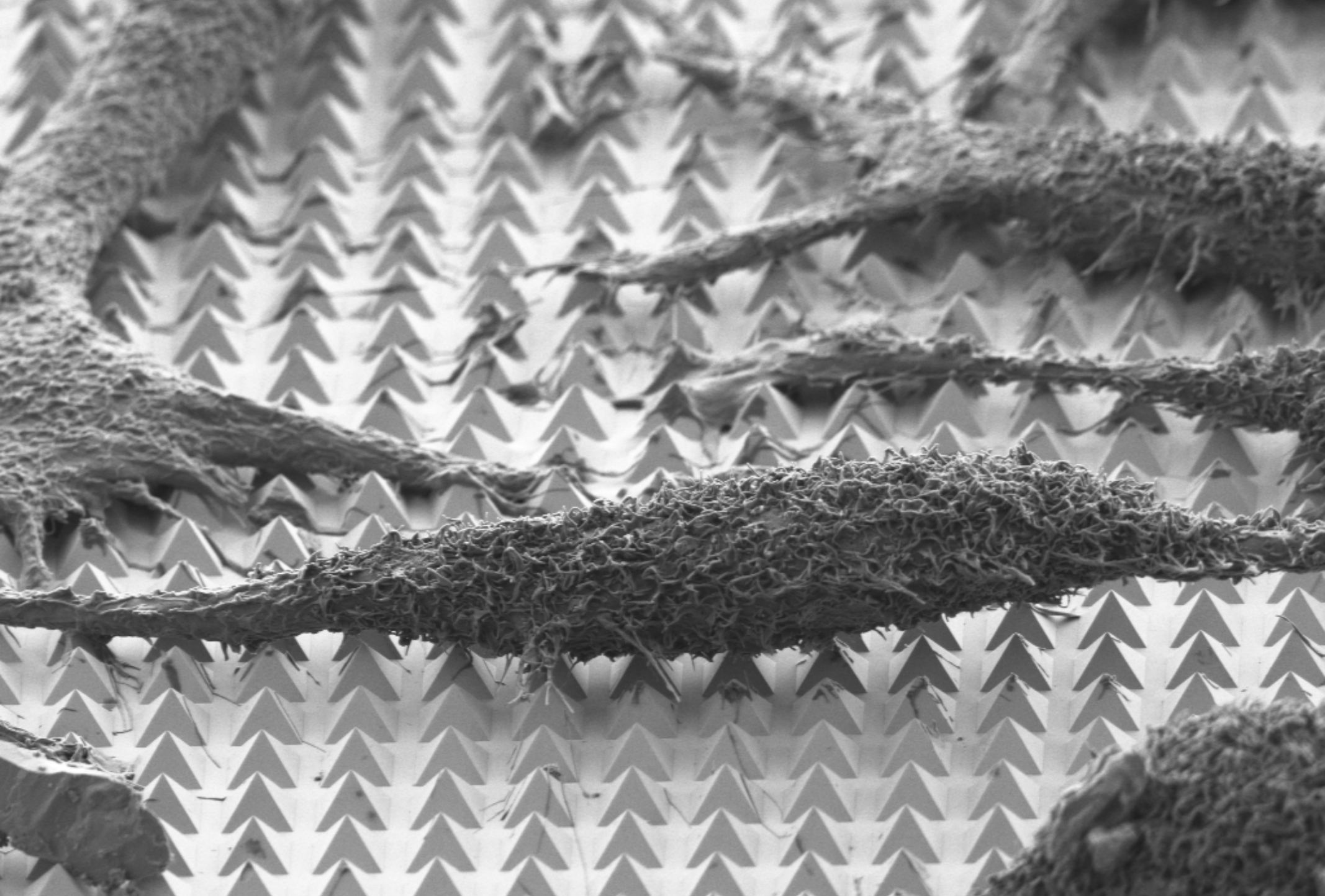


1 substrate



1 substrate

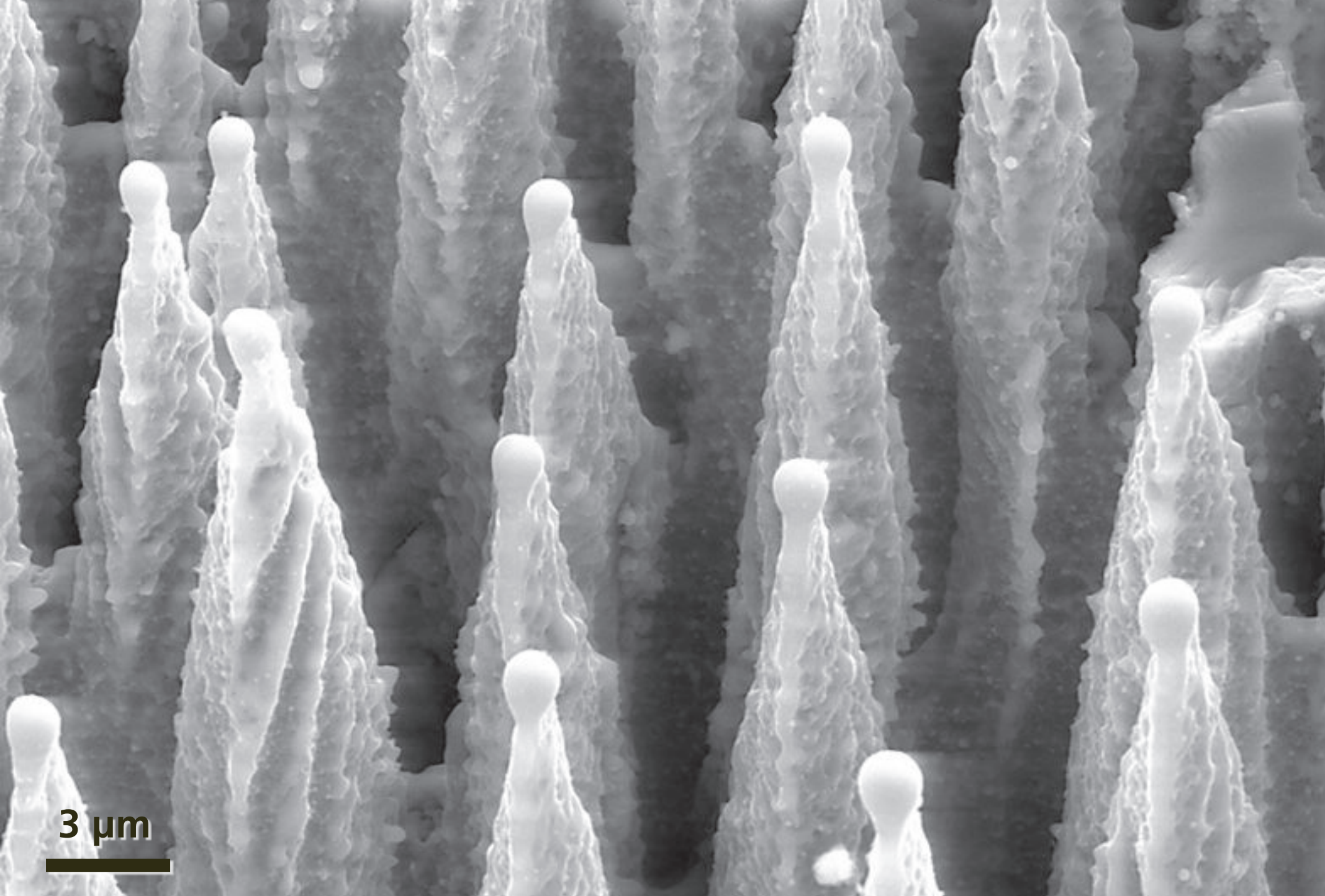
2 mechanism



1 substrate

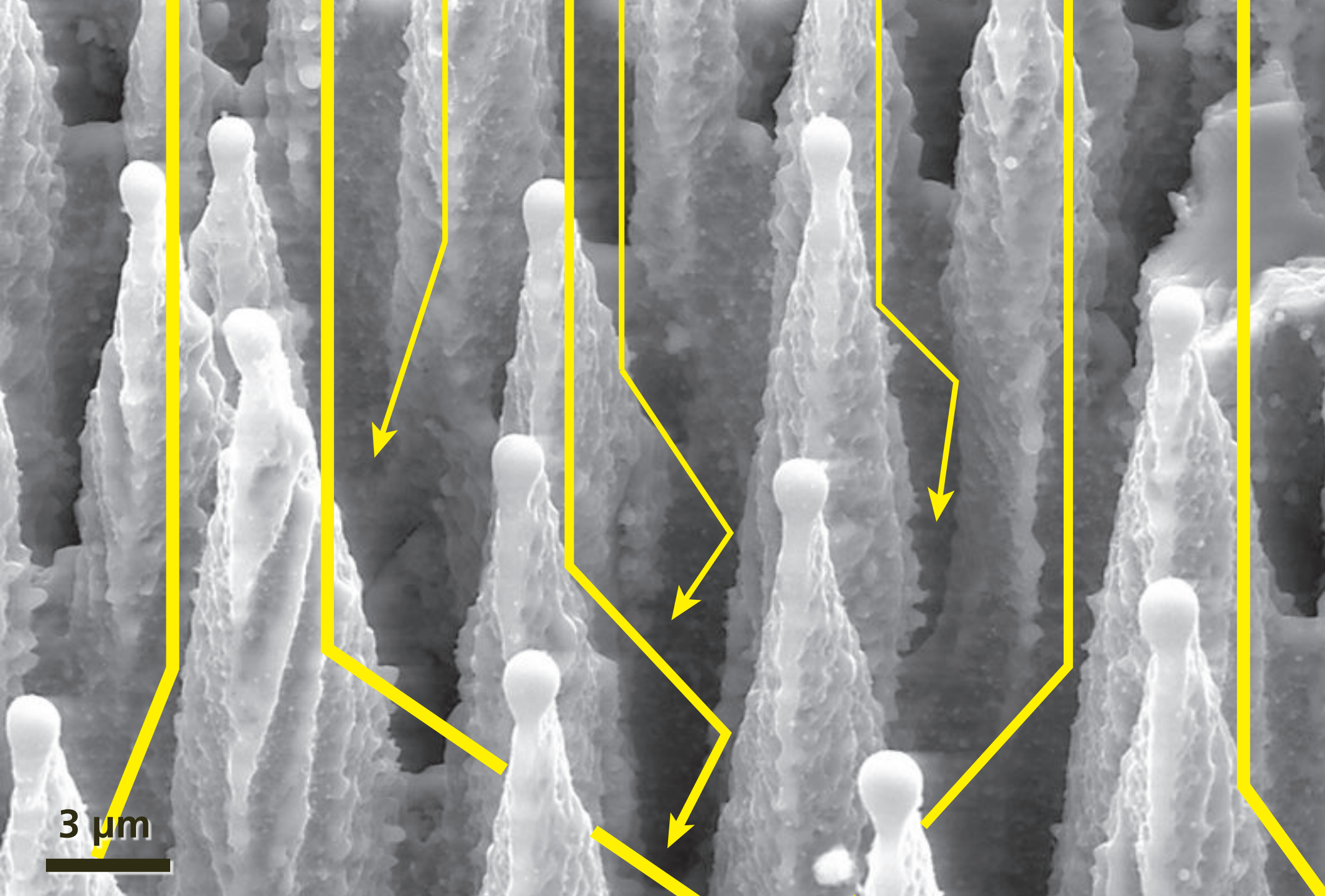
2 mechanism

3 results

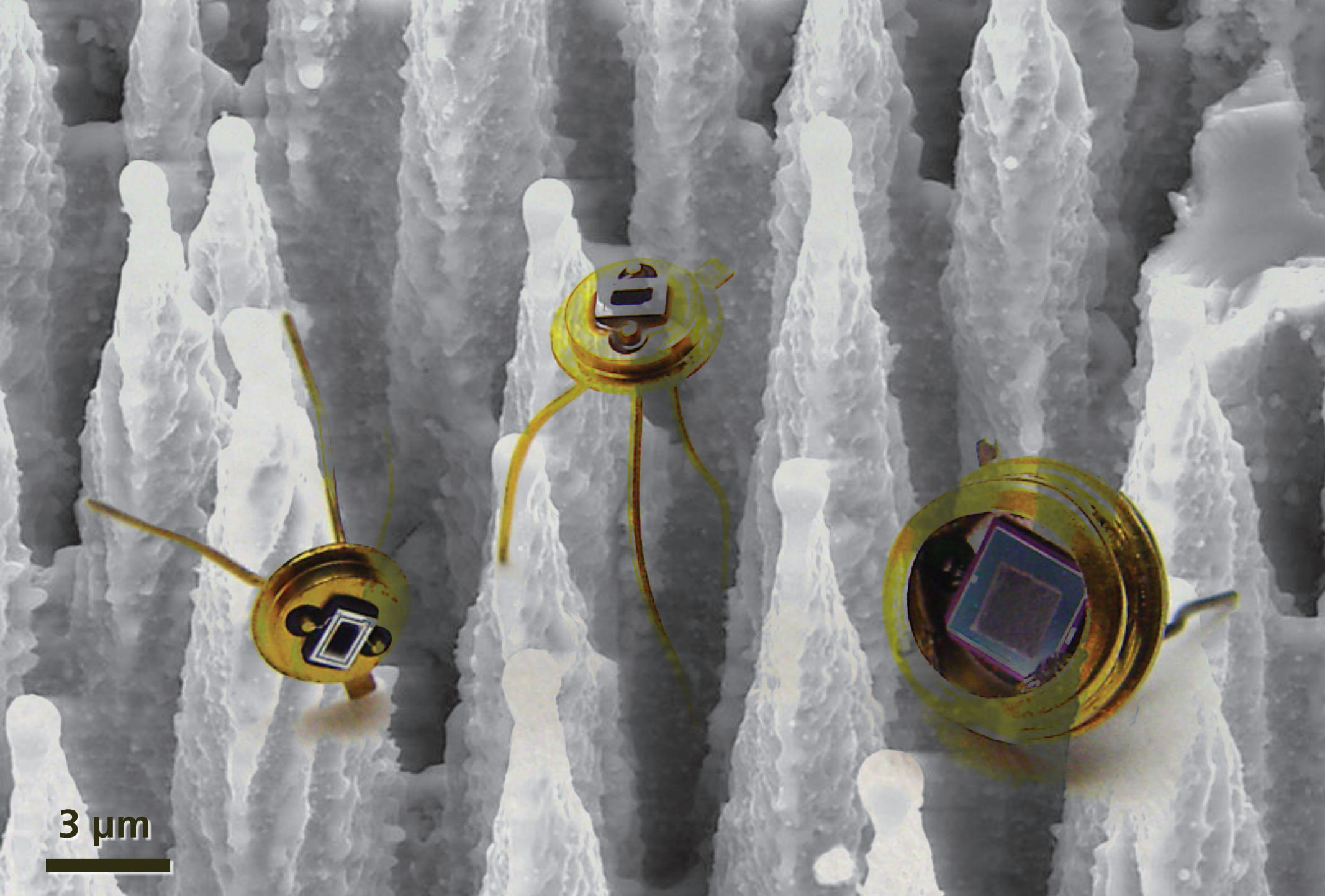


3 μm

1 substrate

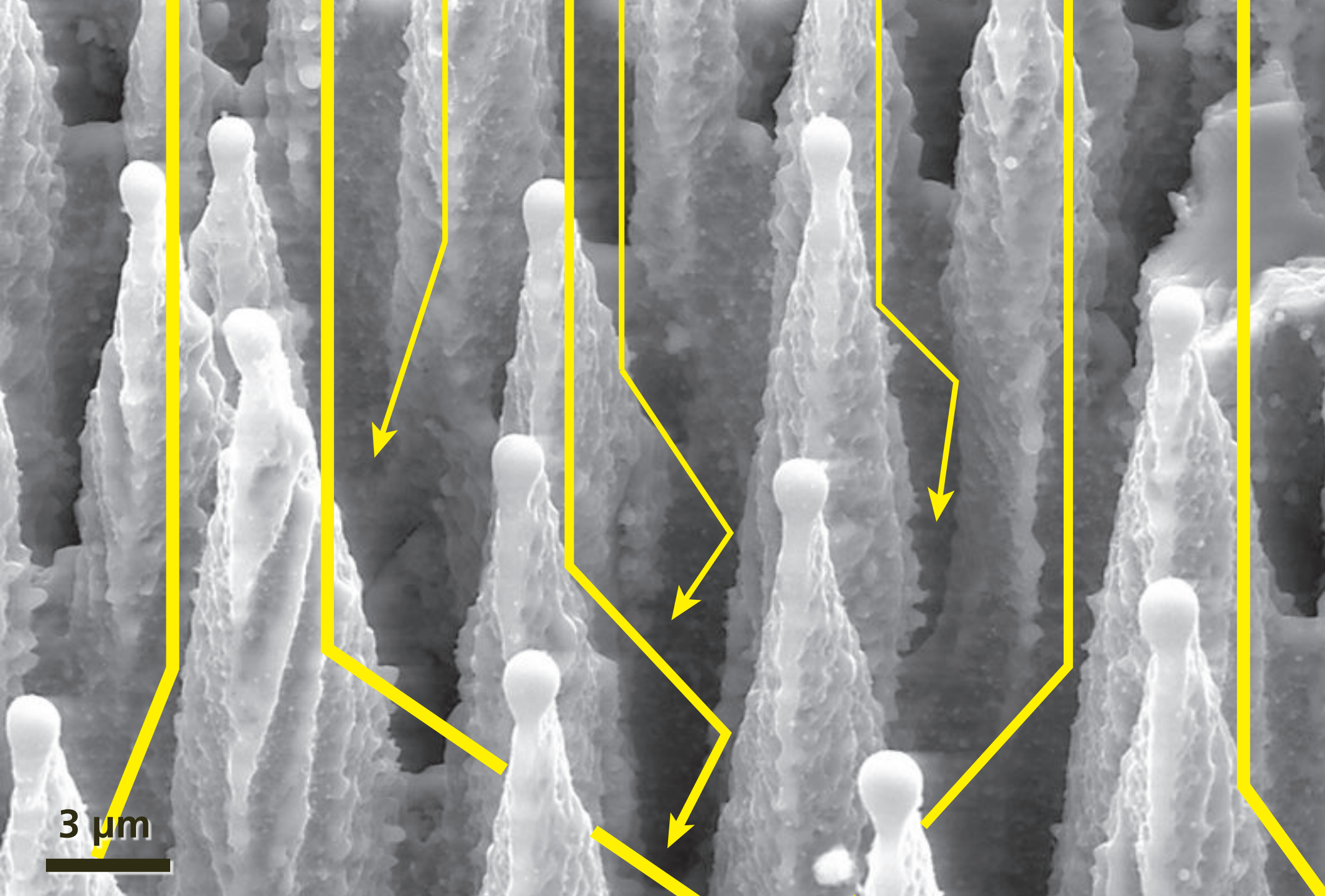


1 substrate

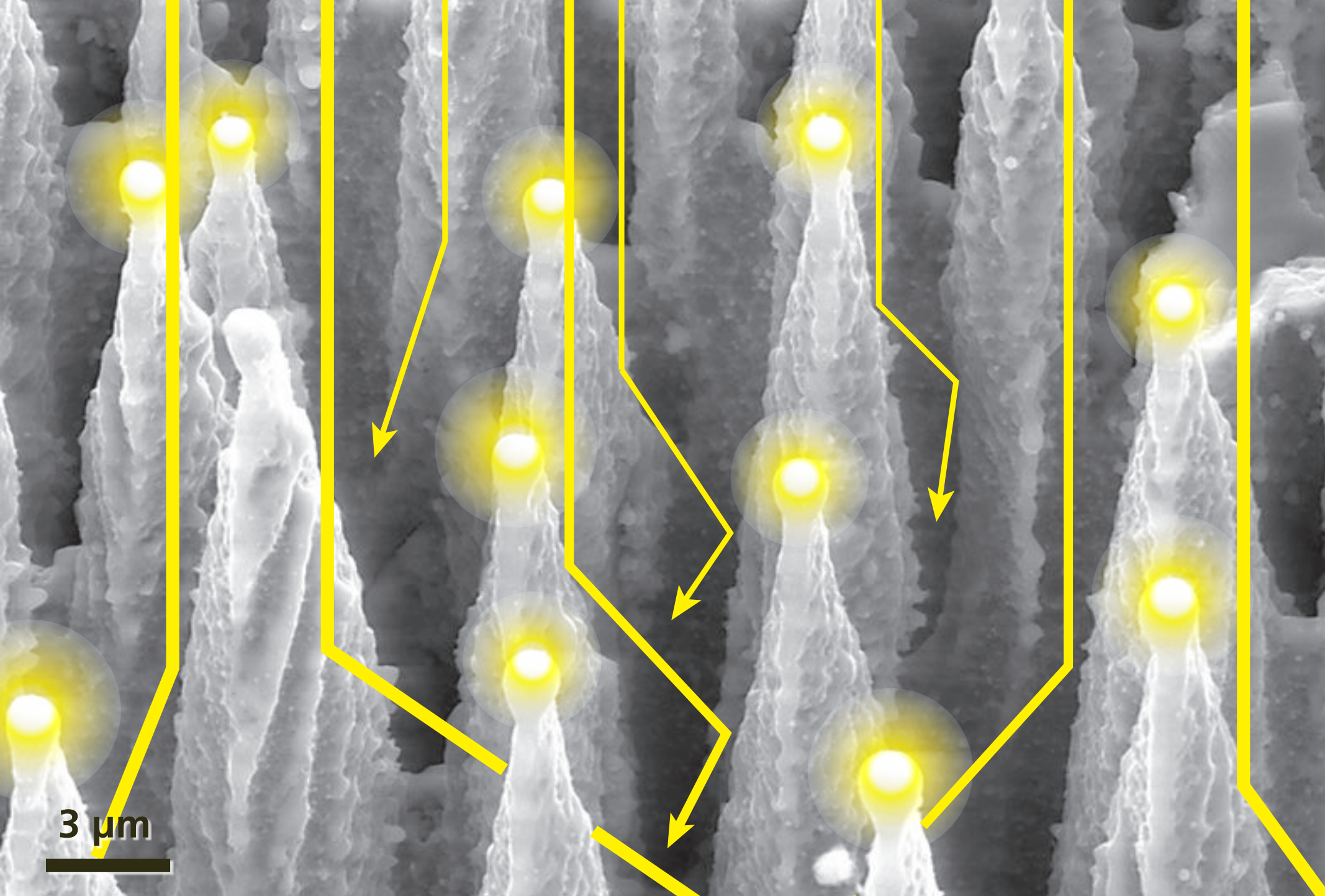


3 μm

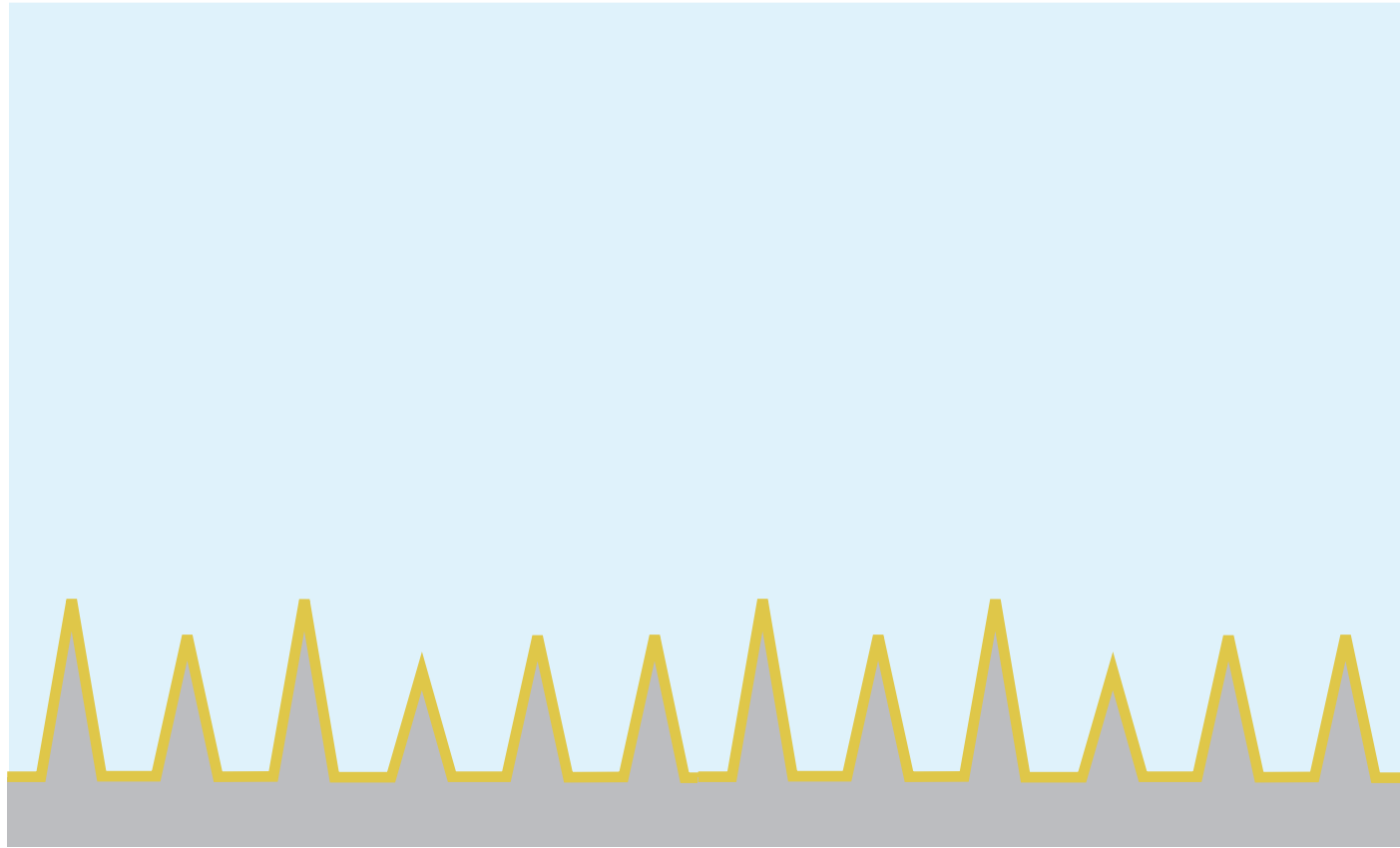
1 substrate



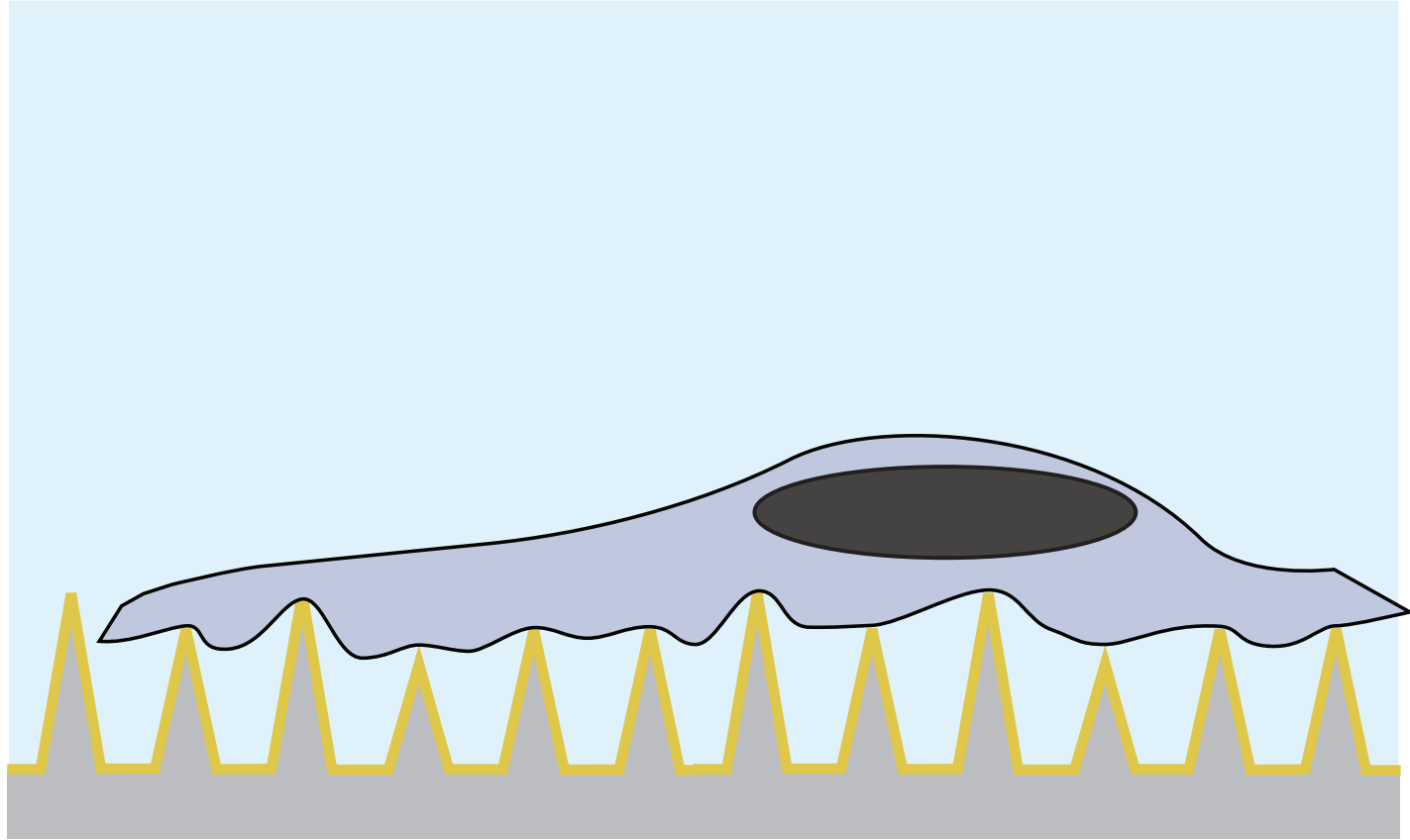
1 substrate



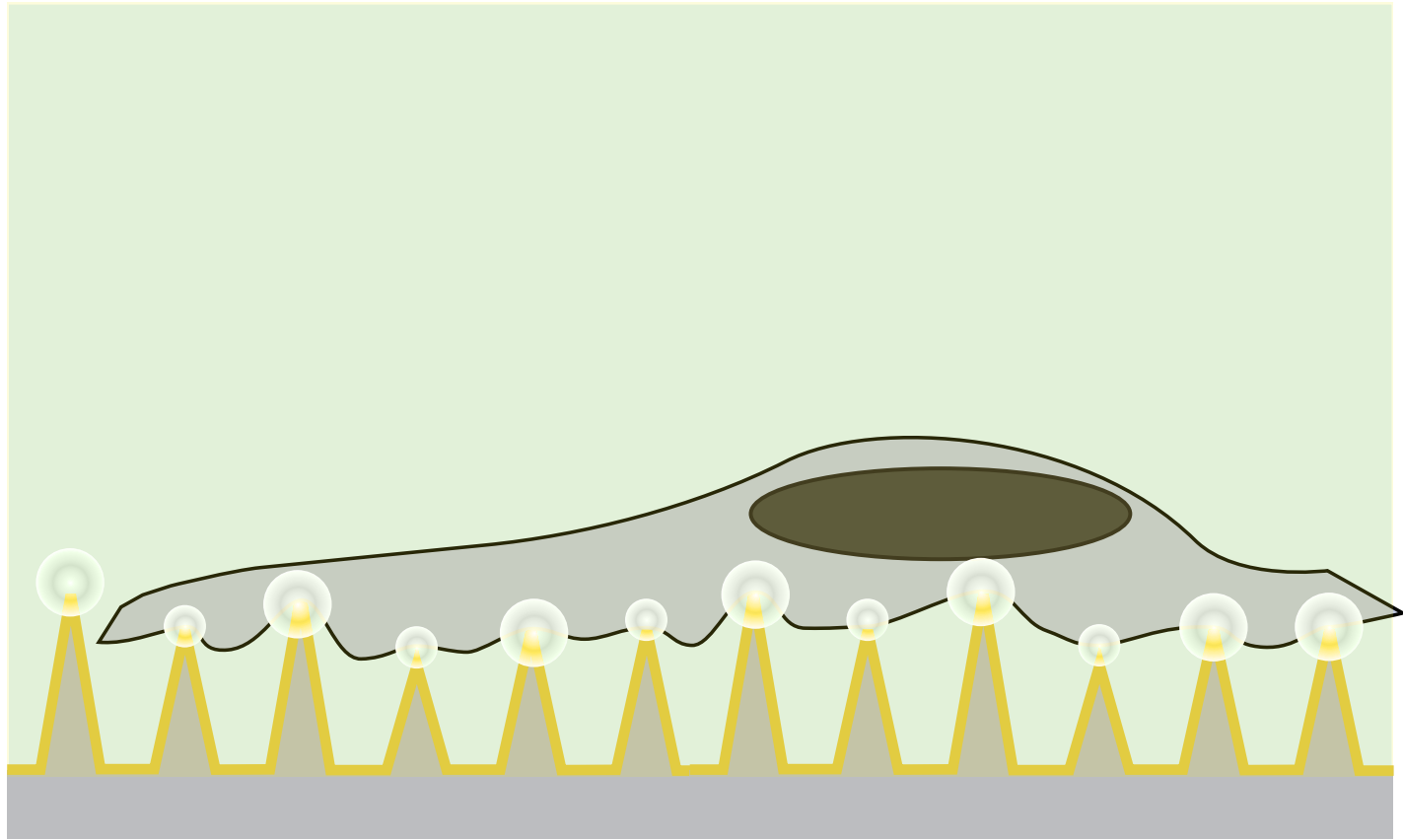
1 substrate



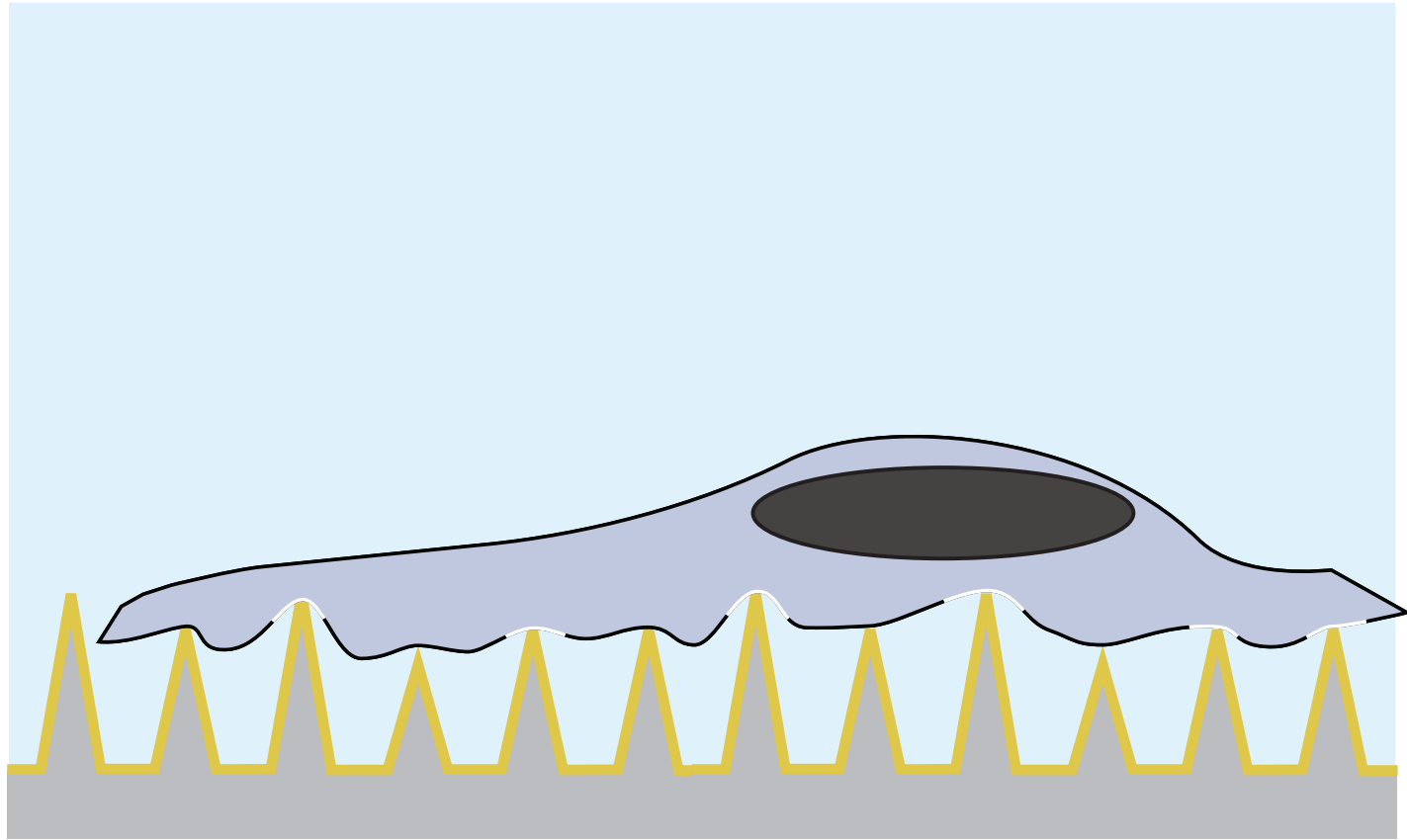
1 substrate



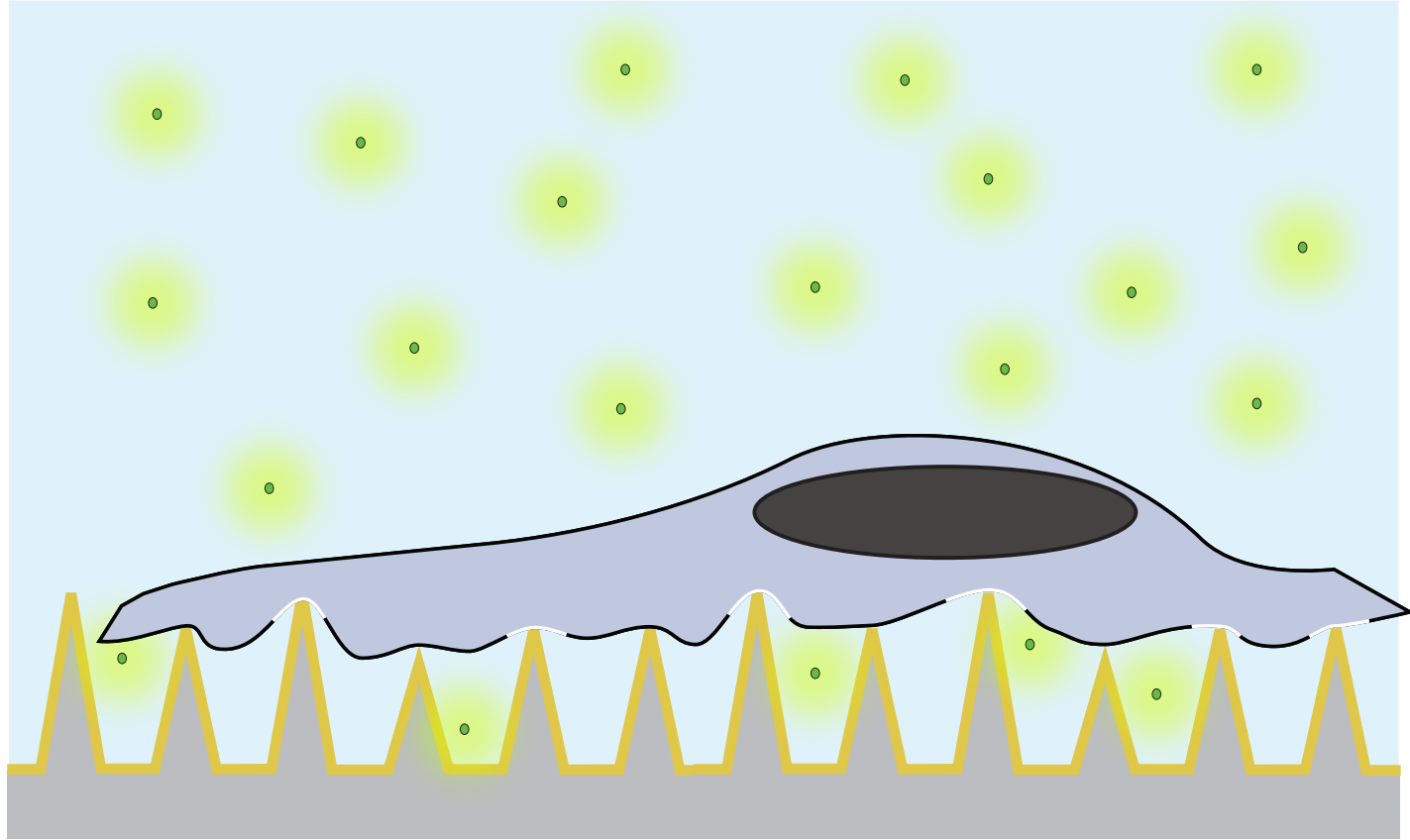
1 substrate



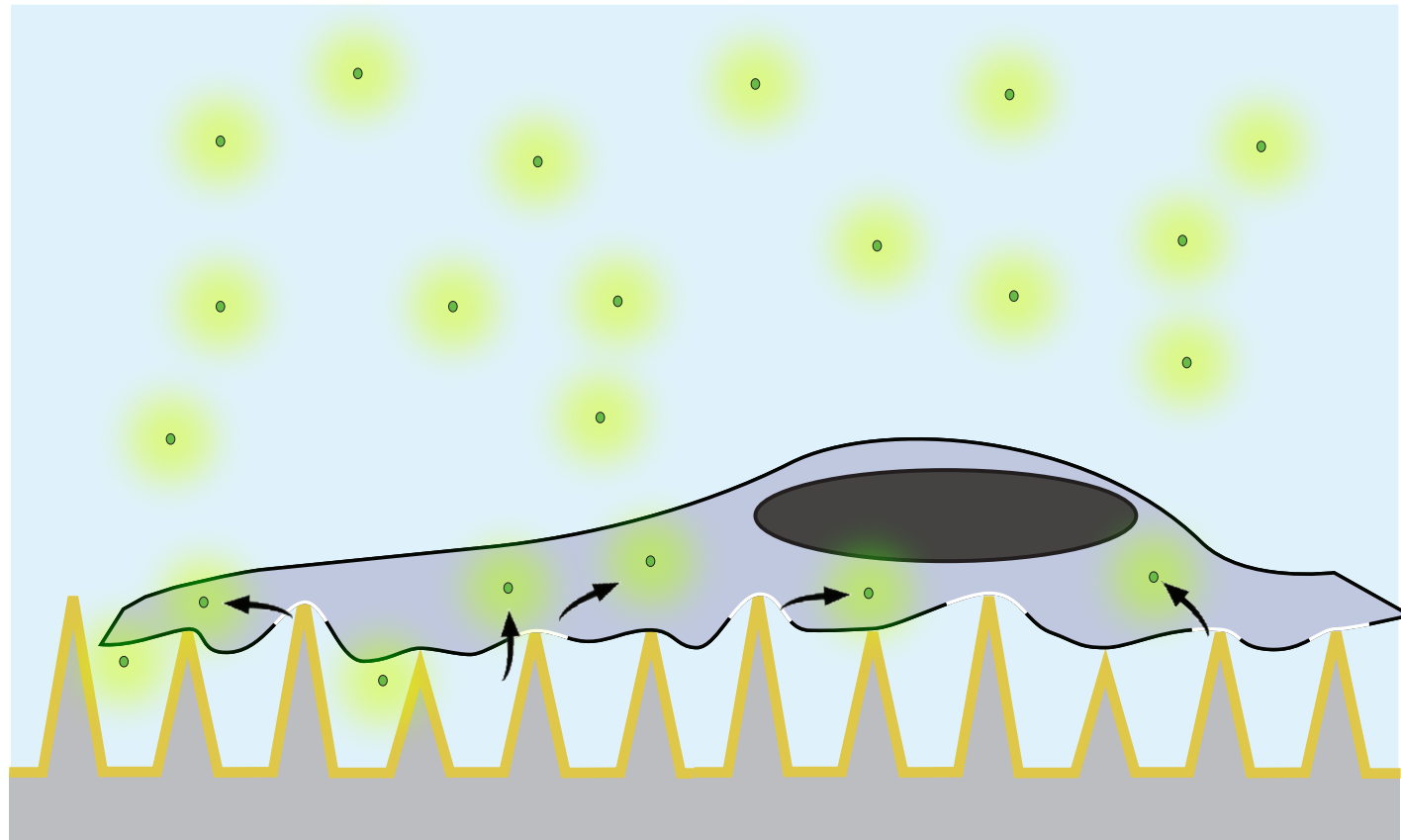
1 substrate



1 substrate



1 substrate



1 substrate



works, but not consistently

solution: engineer substrates



Si

solution: engineer substrates

photoresist



Si

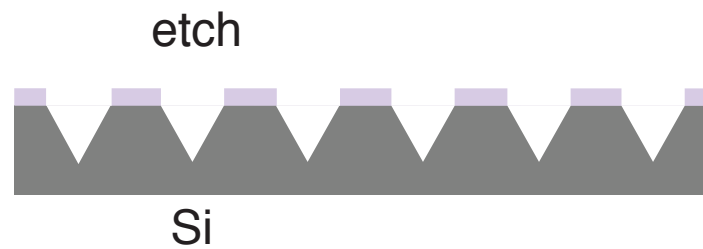
solution: engineer substrates

pattern

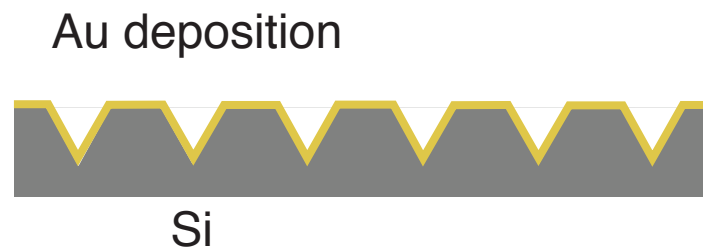


Si

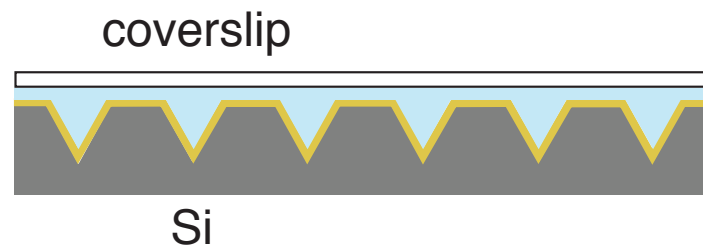
solution: engineer substrates



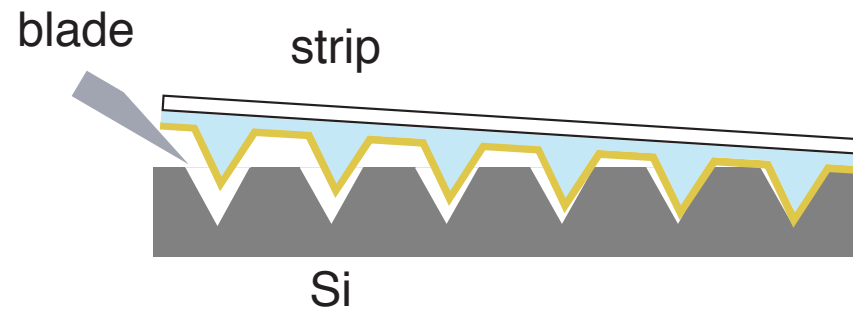
solution: engineer substrates



solution: engineer substrates

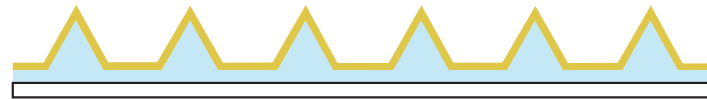


solution: engineer substrates

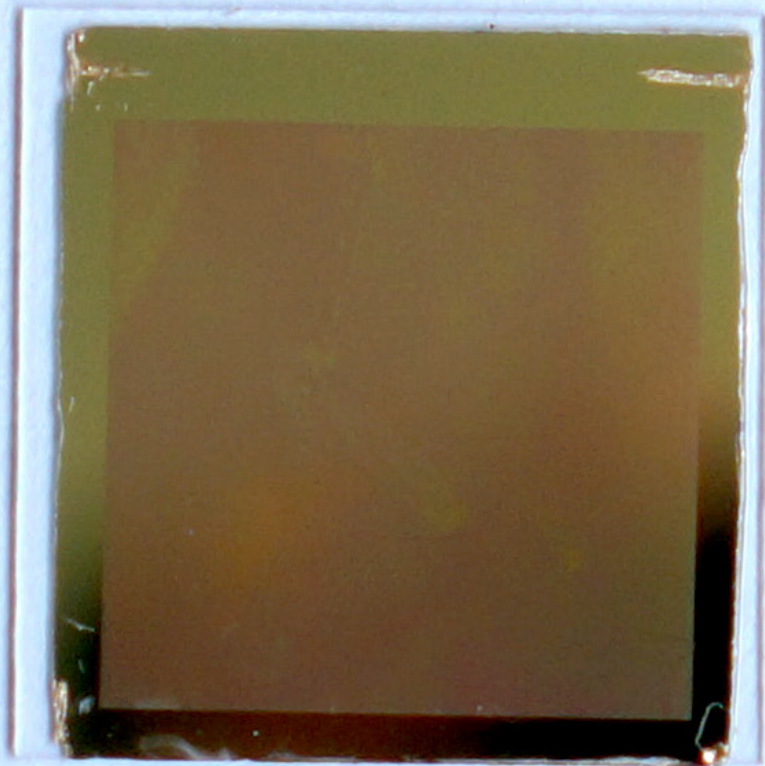


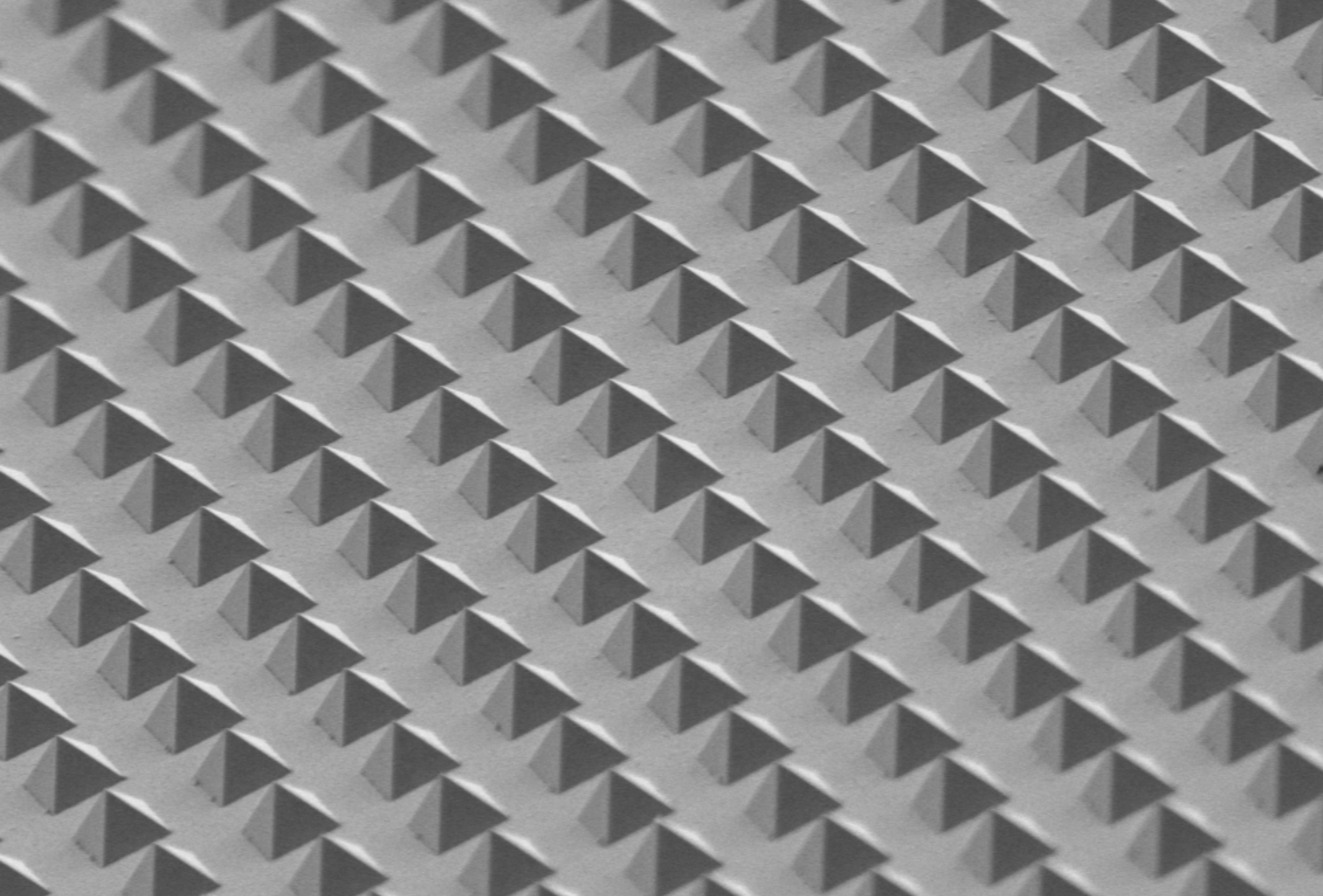
solution: engineer substrates

pyramid substrate



Si





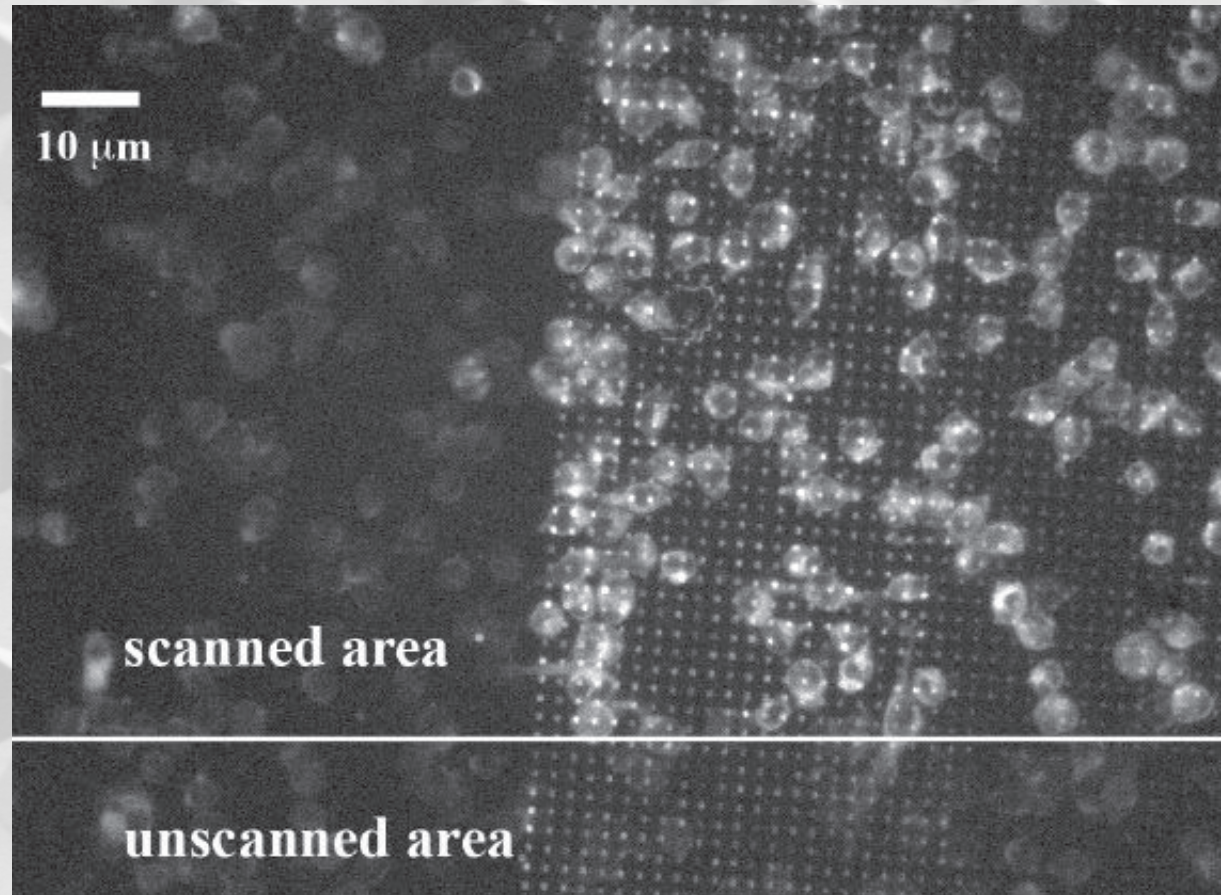
1 substrate

base: 2.4 μm

height: 1.4 μm

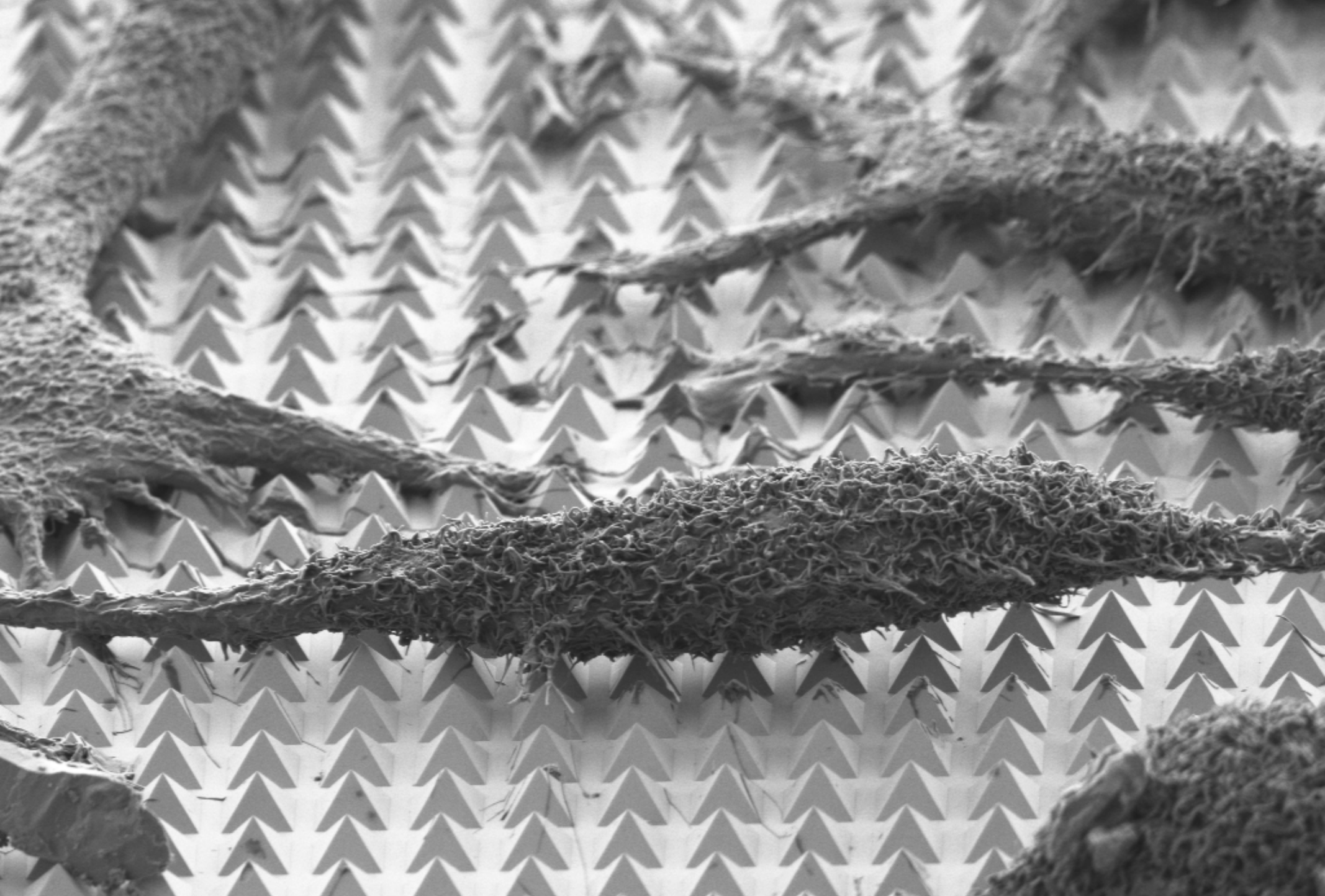
spacing: 3.8 μm

only exposed cells on pyramids take up dye



Diebold, PhD Thesis, Harvard University (2010)

can porate with plasmonic substrates!



1 substrate

2 mechanism

The background of the slide is a light gray color with a repeating pattern of small, three-dimensional cubes. Each cube is oriented with one face pointing towards the viewer, creating a textured, isometric effect.

femtosecond pulses

1 substrate

2 mechanism



femtosecond pulses

1 substrate

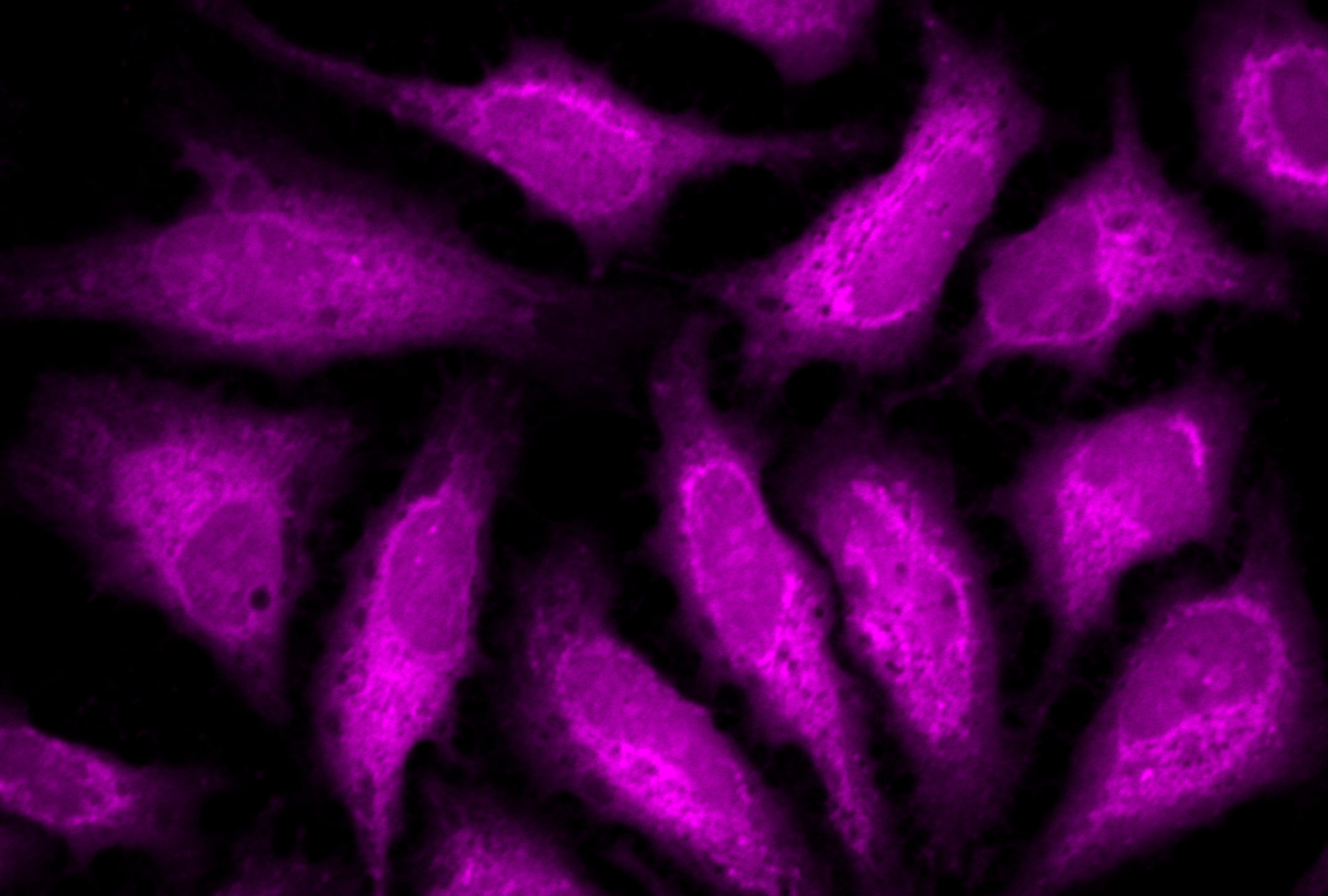
2 mechanism



nanosecond pulses?

1 substrate

2 mechanism



1 substrate

2 mechanism

A microscopic image of cells, possibly bacteria or fungi, showing internal structures and a purple glow. The text "works too!" is overlaid in the center.

works too!

1 substrate

2 mechanism

what mechanism?

plasmons

1 substrate

2 mechanism

what mechanism?

plasmons



NF enhancement

what mechanism?

plasmons

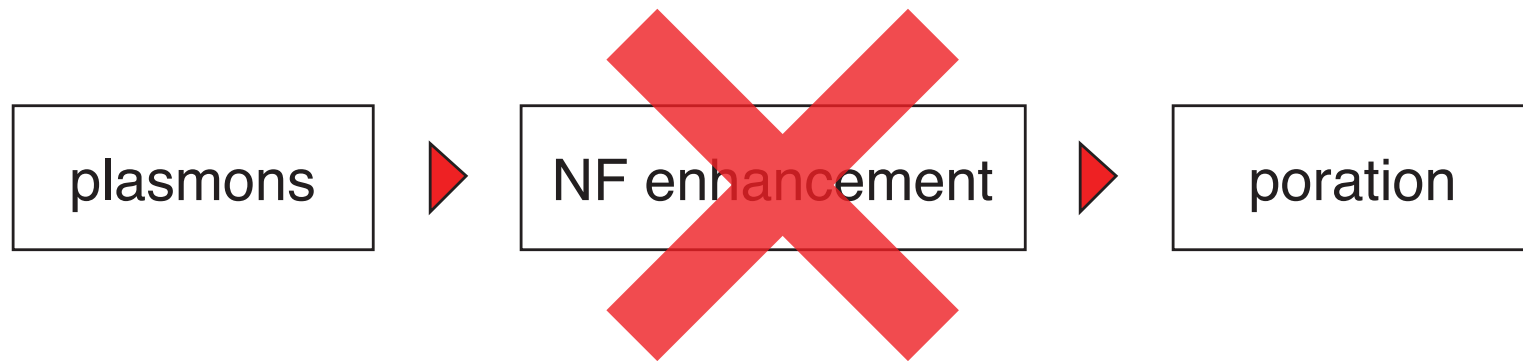


NF enhancement



poration

what mechanism?



1 substrate

2 mechanism

what mechanism?

plasmons



heating

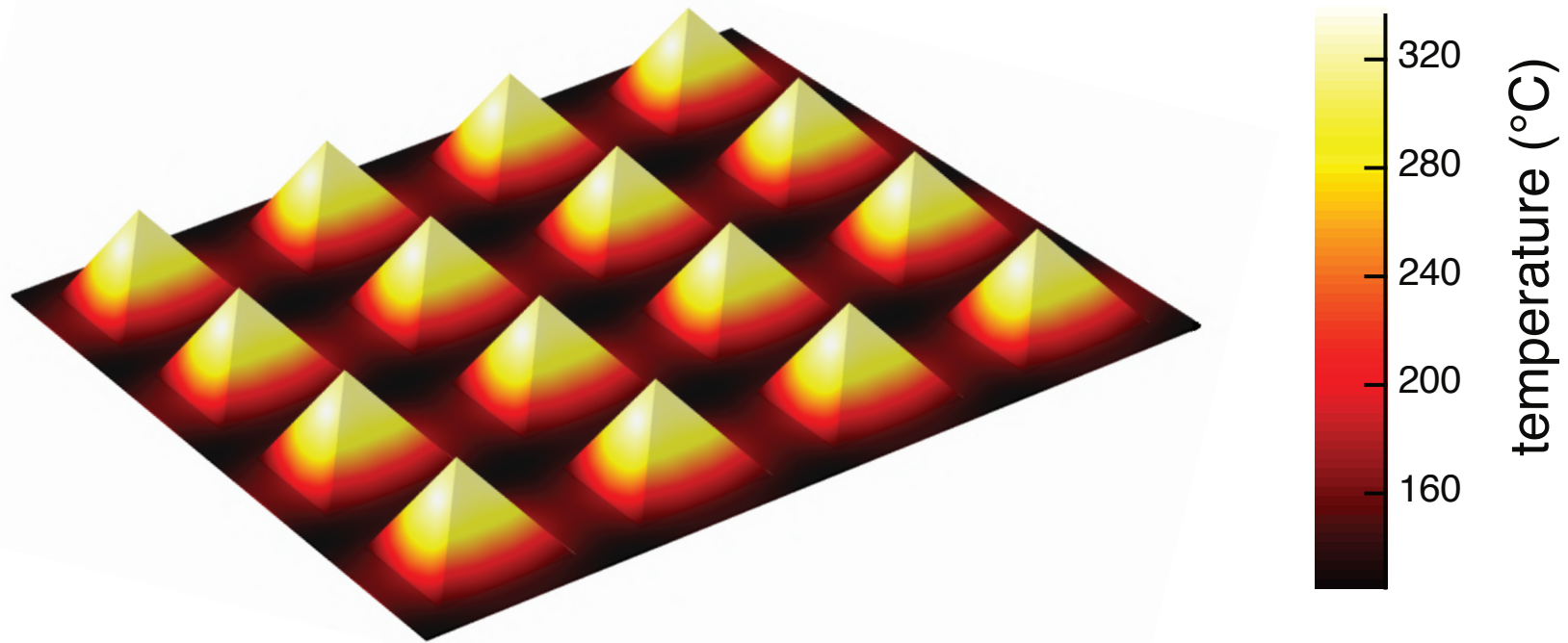


poration

1 substrate

2 mechanism

finite element modeling

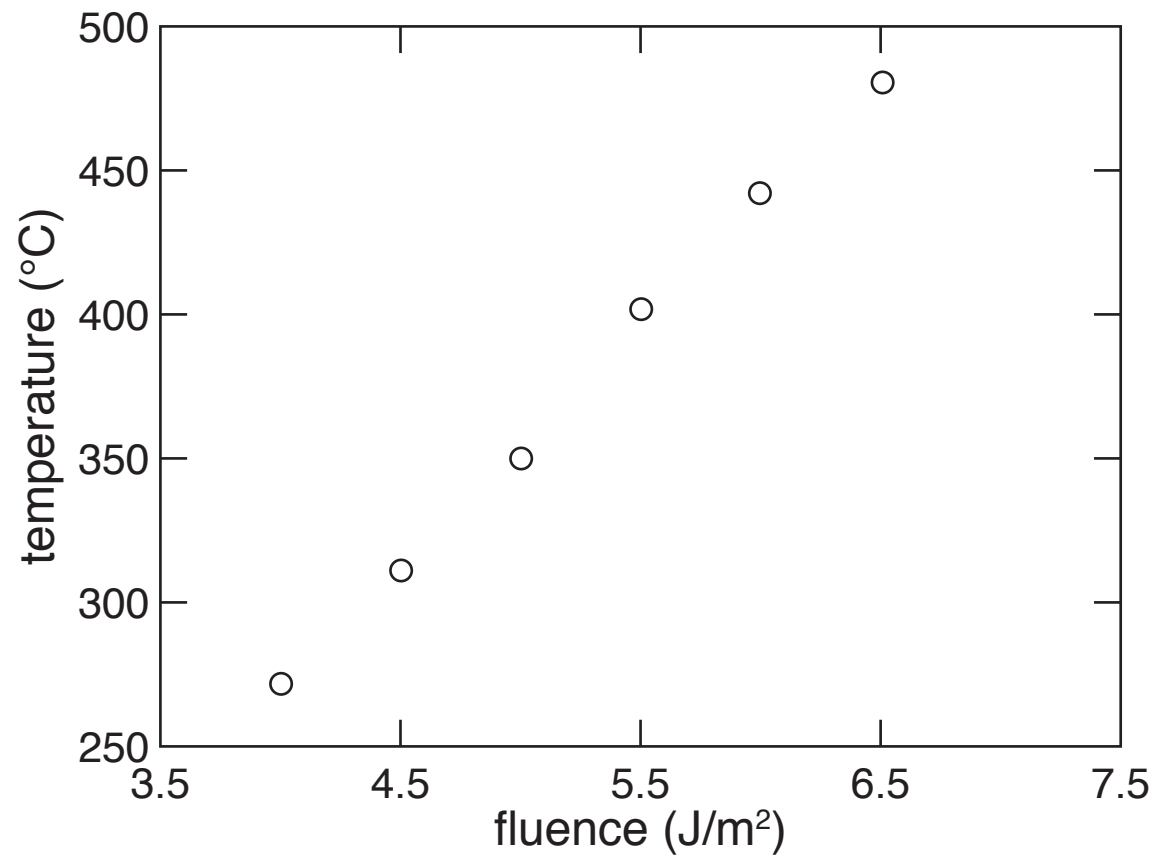


Huber *et al.*, submitted for publication

1 substrate

2 mechanism

surface temperature

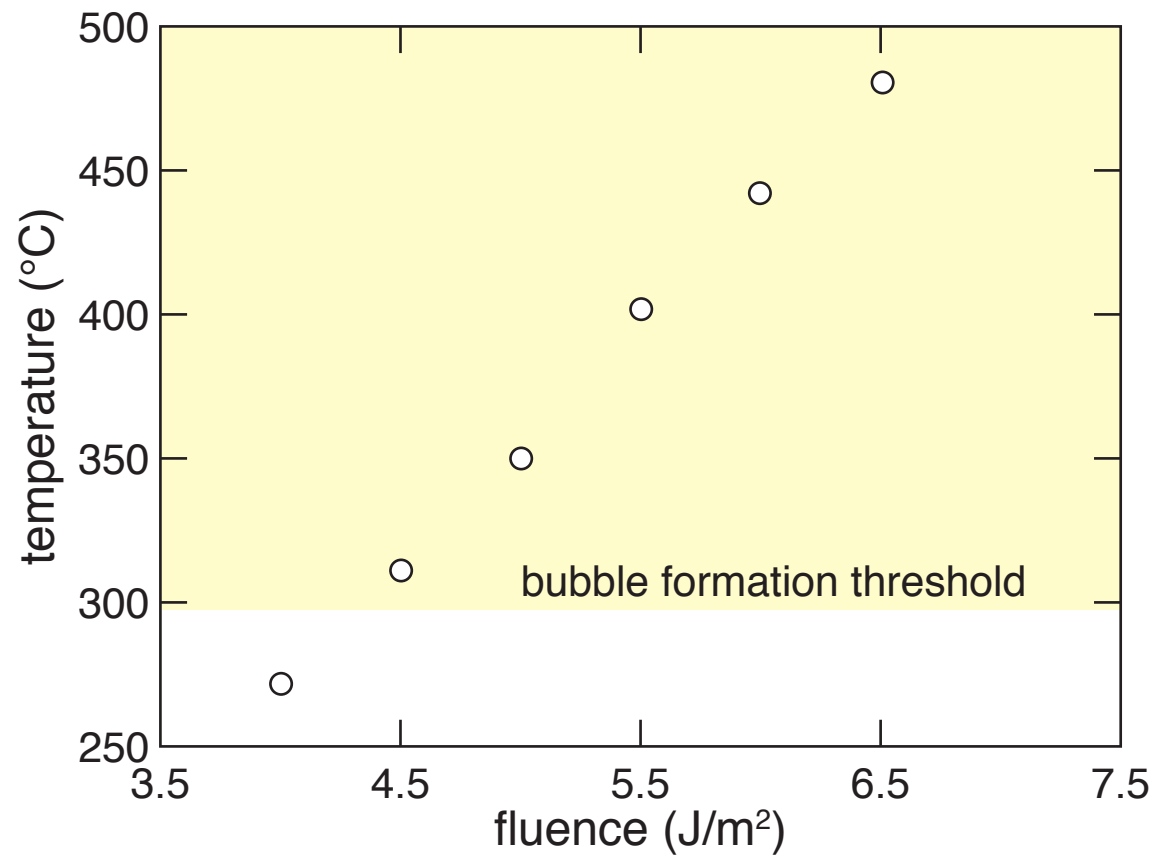


Huber *et al.*, submitted for publication

1 substrate

2 mechanism

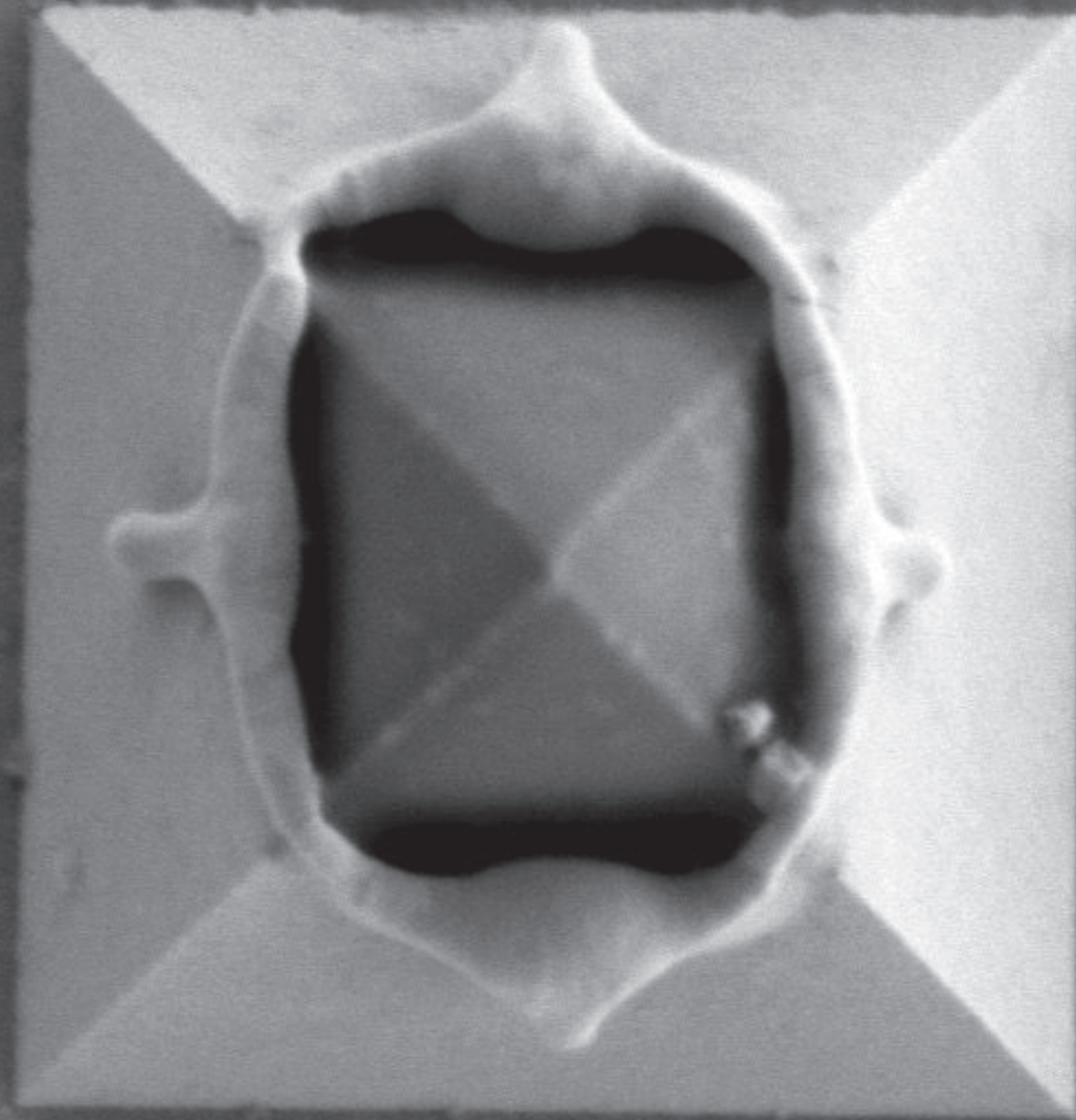
surface temperature



Huber *et al.*, submitted for publication

1 substrate

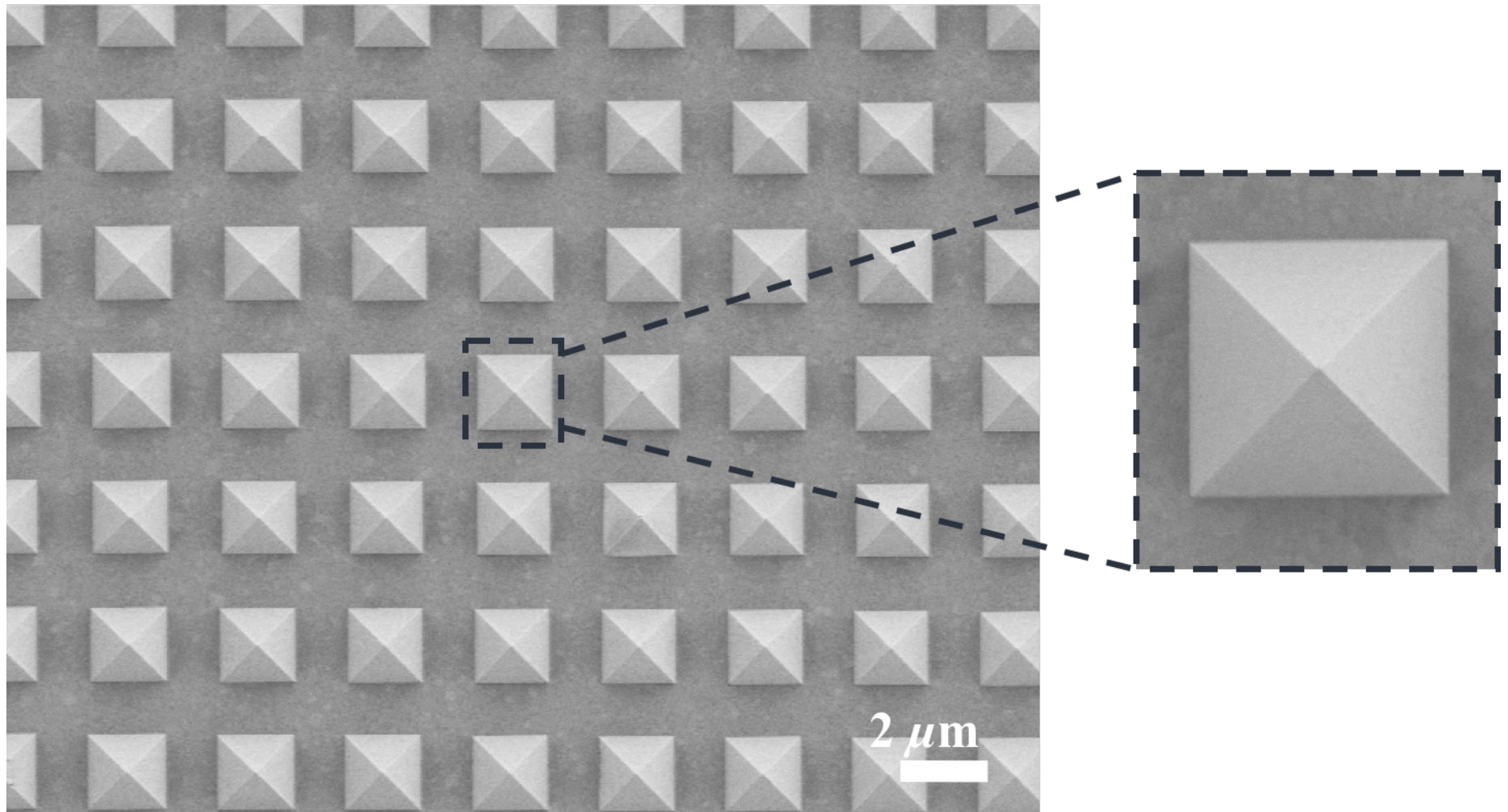
2 mechanism



$> 20 \text{ J/m}^2$

① substrate

② mechanism

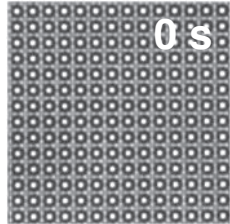


5.4 J/m^2

1 substrate

2 mechanism

bubble formation

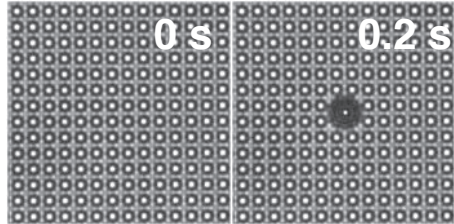


Chen *et al.*, submitted for publication

1 substrate

2 mechanism

bubble formation

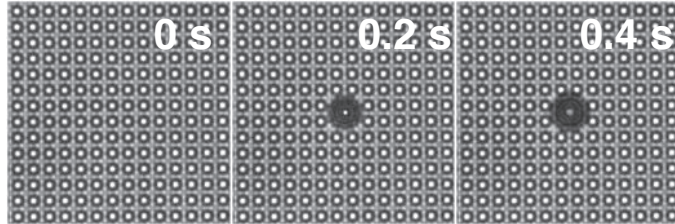


Chen *et al.*, submitted for publication

1 substrate

2 mechanism

bubble formation

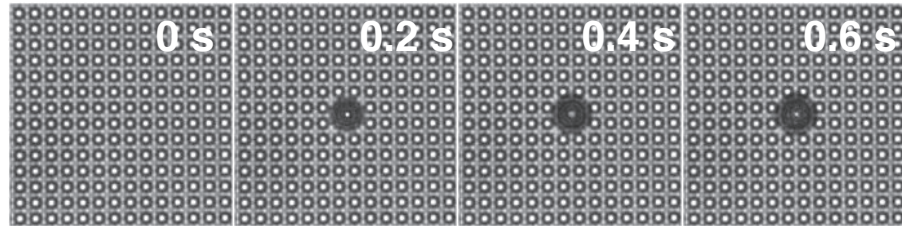


Chen *et al.*, submitted for publication

1 substrate

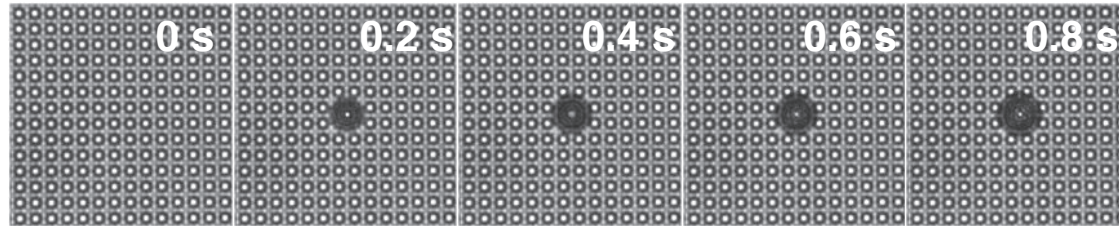
2 mechanism

bubble formation



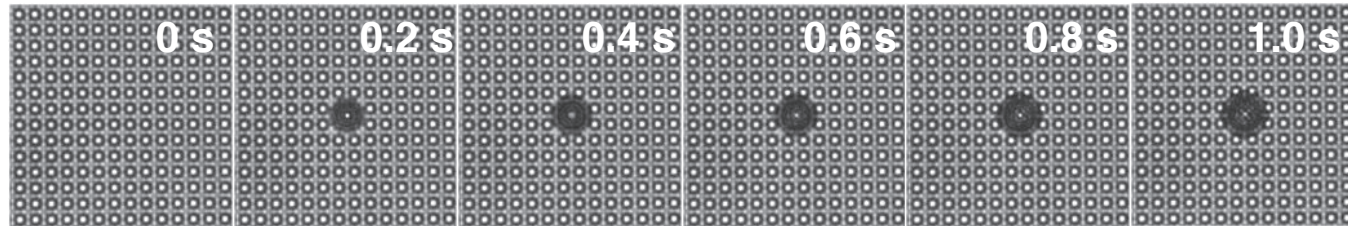
Chen *et al.*, submitted for publication

bubble formation



Chen *et al.*, submitted for publication

bubble formation

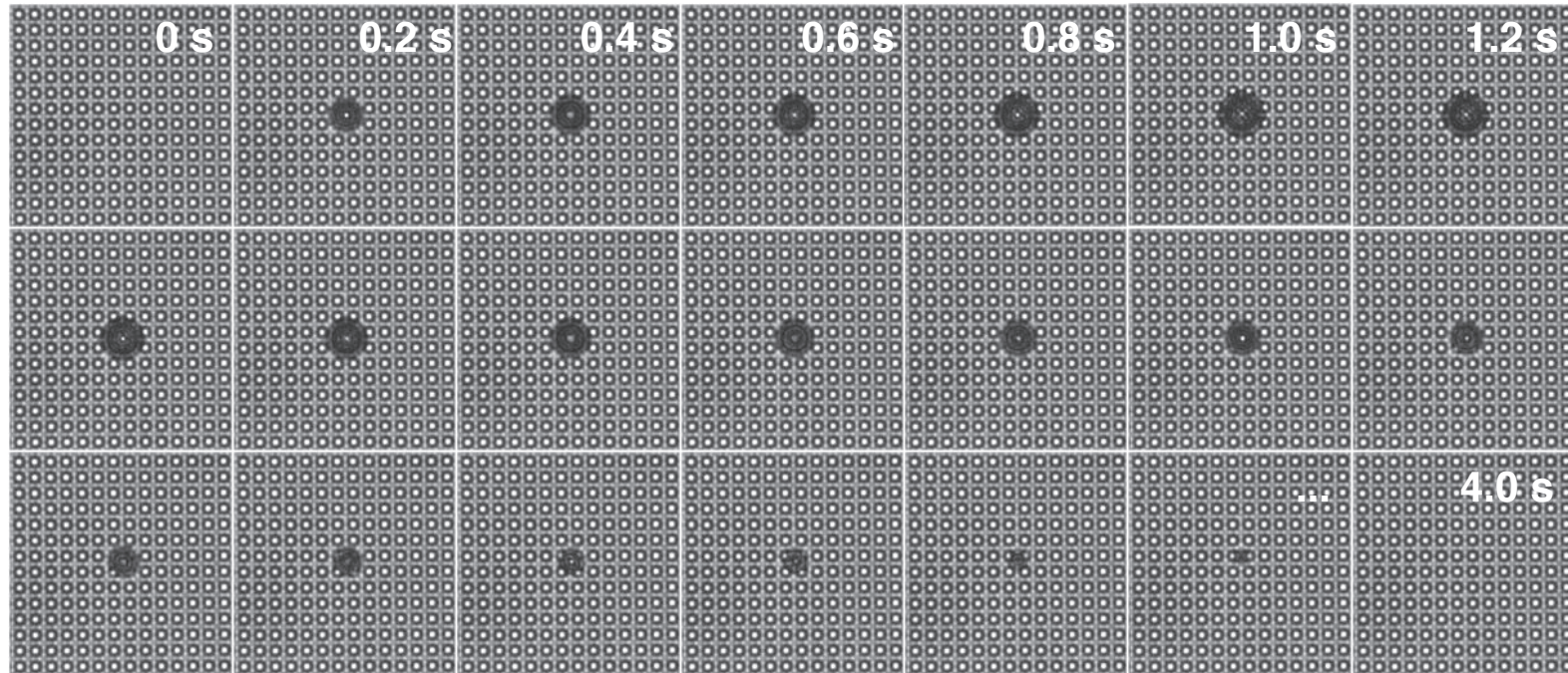


Chen *et al.*, submitted for publication

1 substrate

2 mechanism

bubble formation and collapse



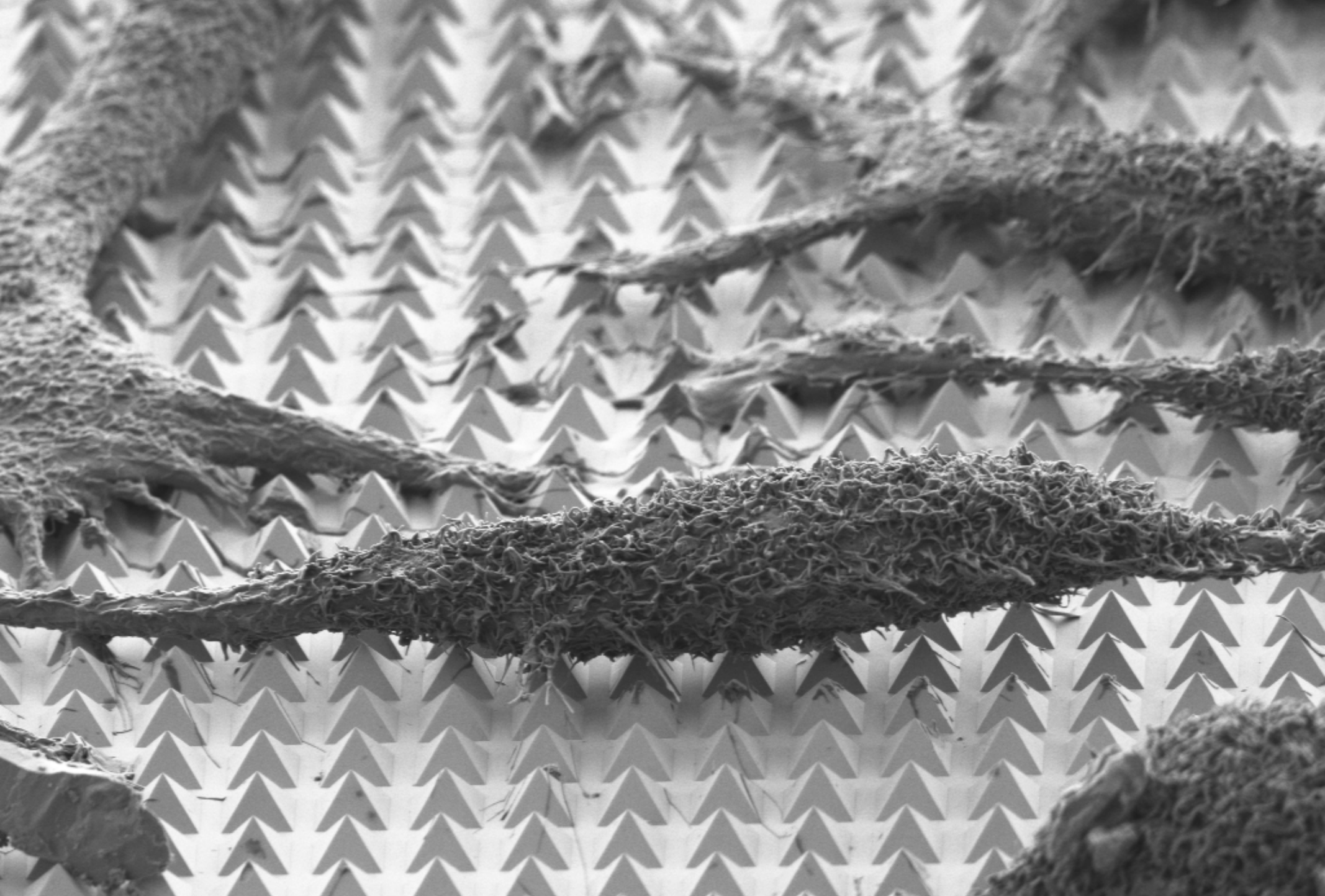
Chen *et al.*, submitted for publication



poration mediated by microbubbles

1 substrate

2 mechanism



1 substrate

2 mechanism

3 results

goal

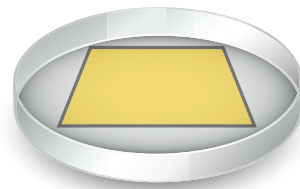
Viability	Efficiency	Throughput	Versatility
H	H	H	H

1 substrate

2 mechanism

3 results

experimental protocol

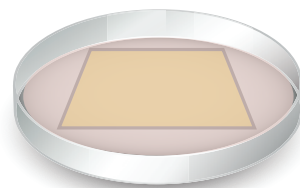


1 substrate

2 mechanism

3 results

experimental protocol



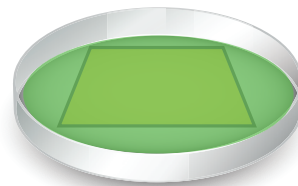
seed HeLa cells

1 substrate

2 mechanism

3 results

experimental protocol



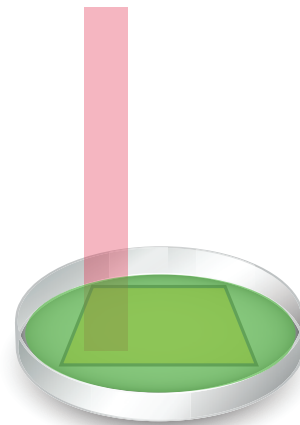
add calcein green

1 substrate

2 mechanism

3 results

experimental protocol



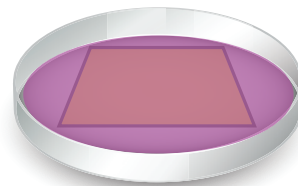
scan laser

1 substrate

2 mechanism

3 results

experimental protocol



viability indicator

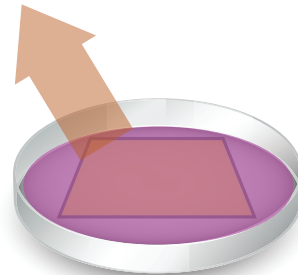
1 substrate

2 mechanism

3 results

experimental protocol

fluorescence
microscopy



viability indicator

1 substrate

2 mechanism

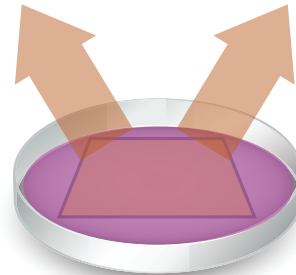
3 results

experimental protocol

fluorescence
microscopy



flow
cytometry



viability indicator

1 substrate

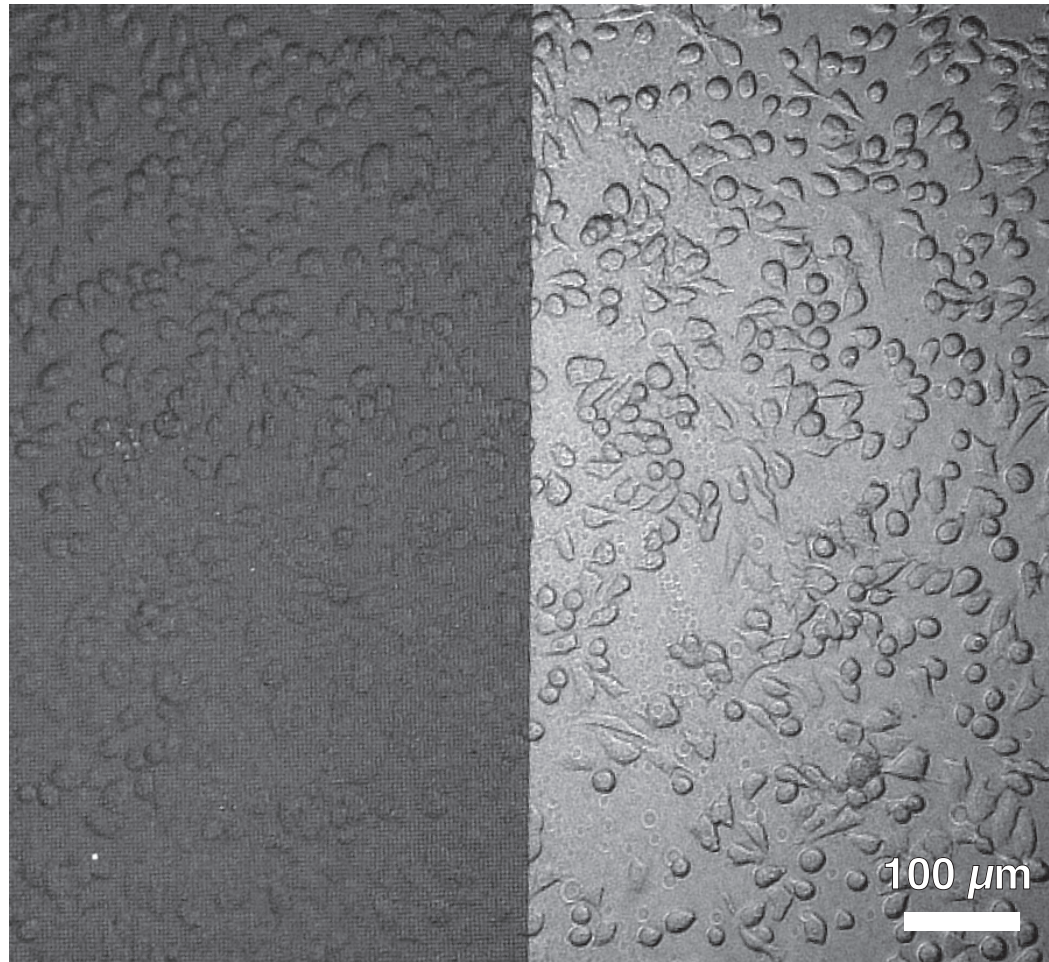
2 mechanism

3 results

fluorescence microscopy

pyramids

flat

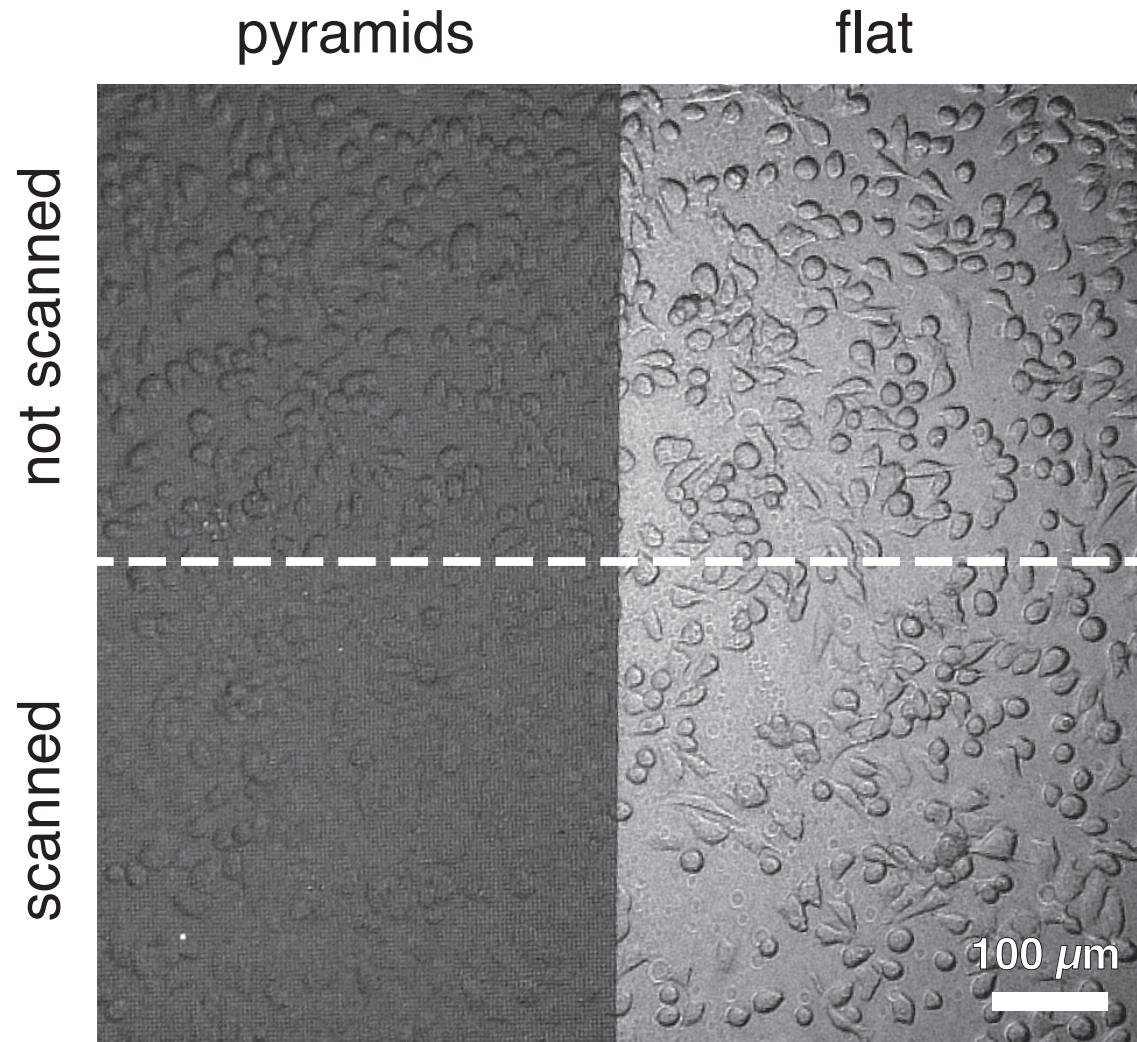


1 substrate

2 mechanism

3 results

fluorescence microscopy

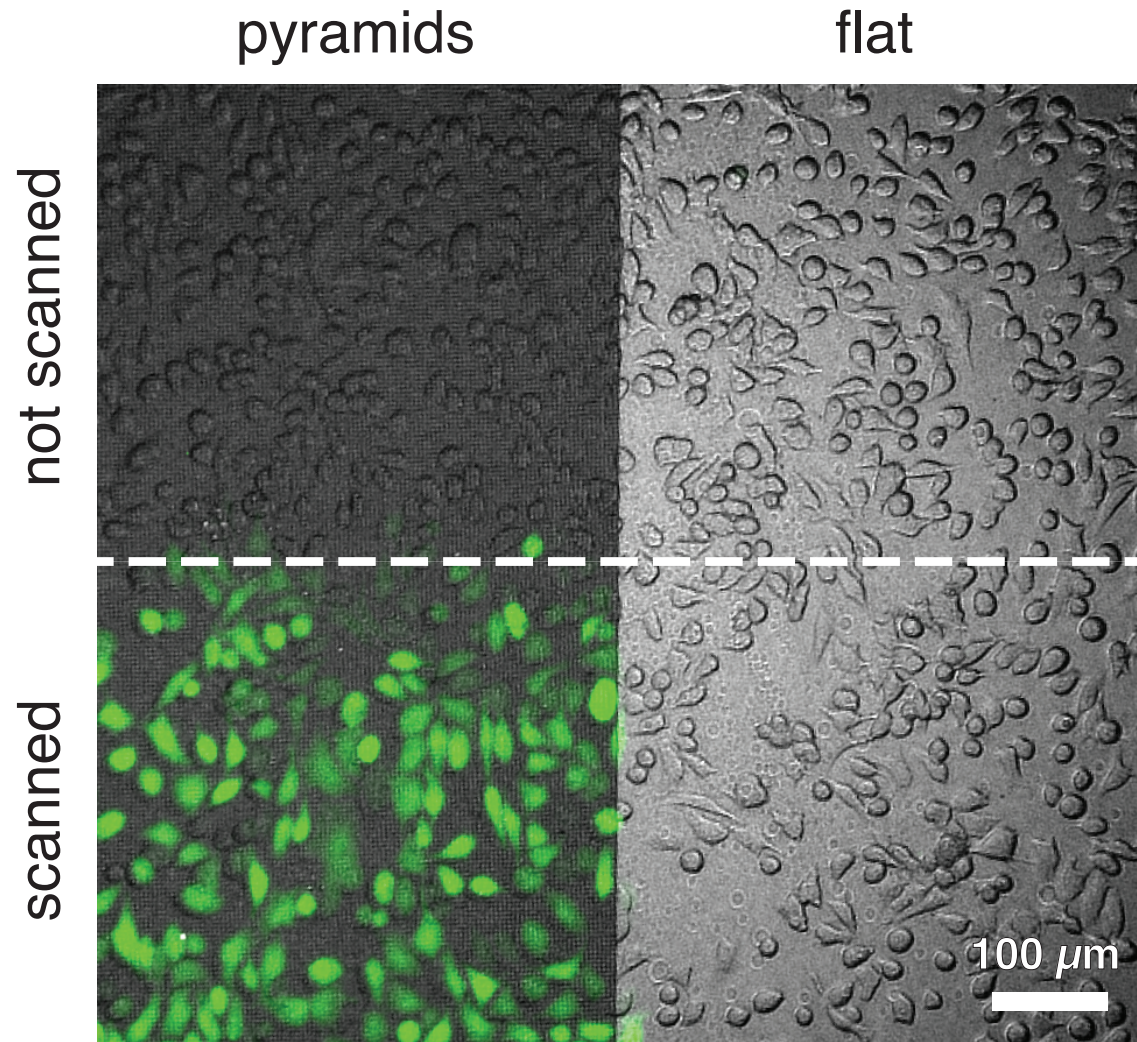


1 substrate

2 mechanism

3 results

fluorescence microscopy



1 substrate

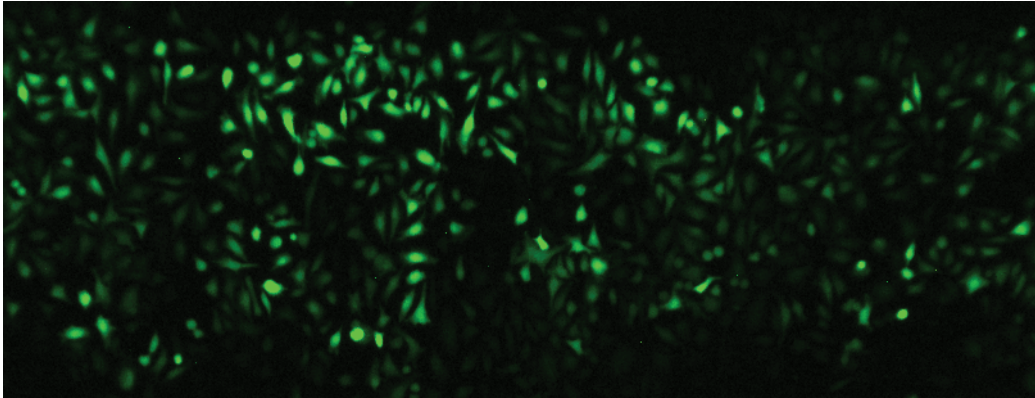
2 mechanism

3 results

fluorescence microscopy

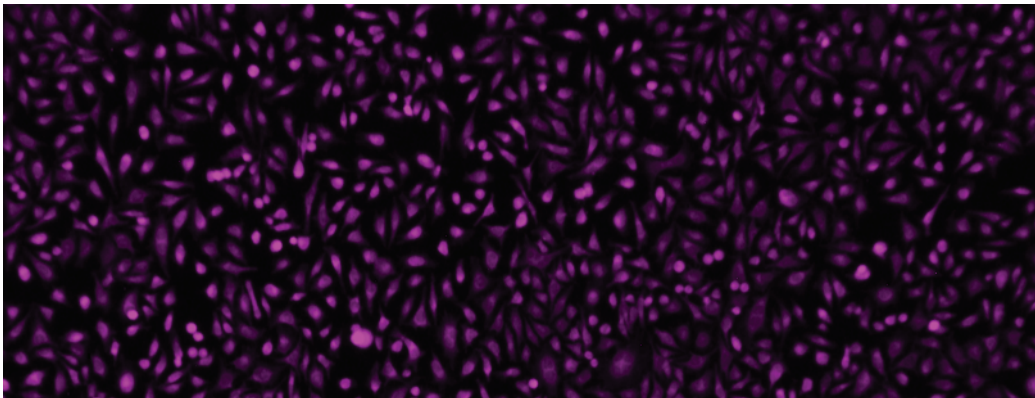
efficiency

calcein green



78% of cells

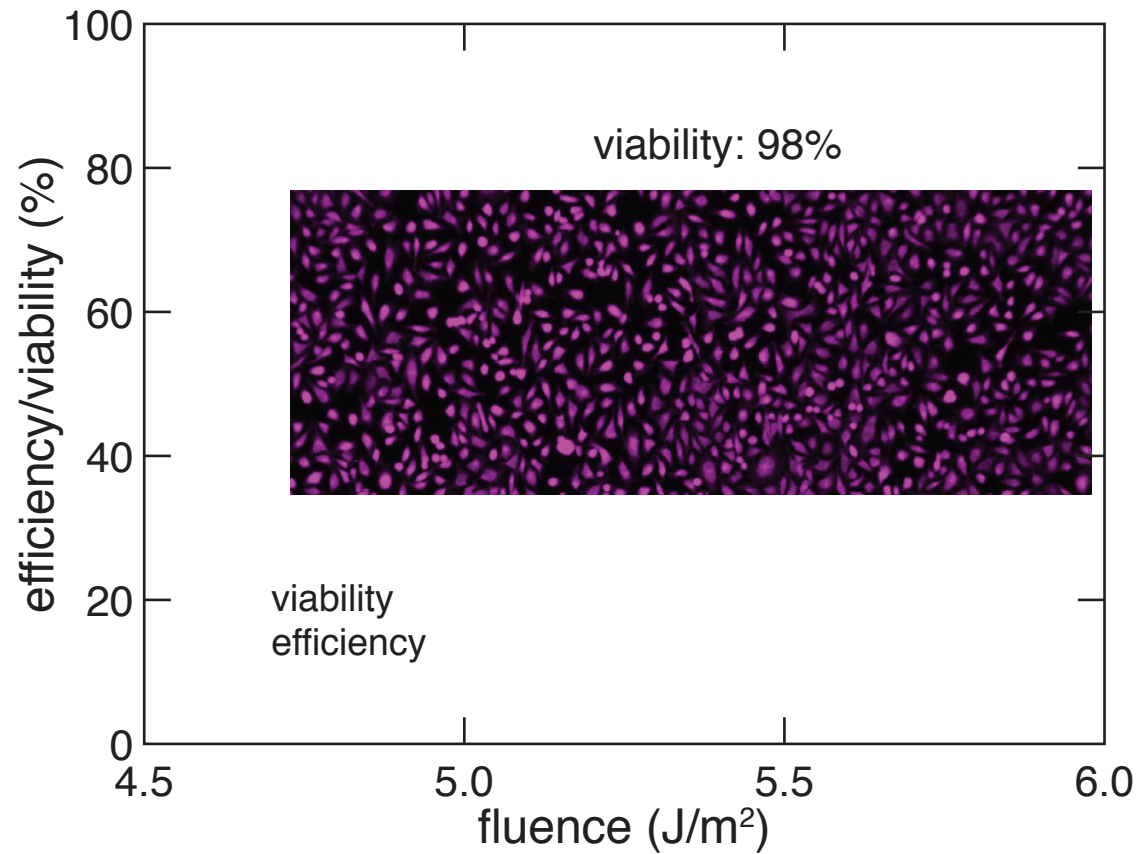
calcein AM



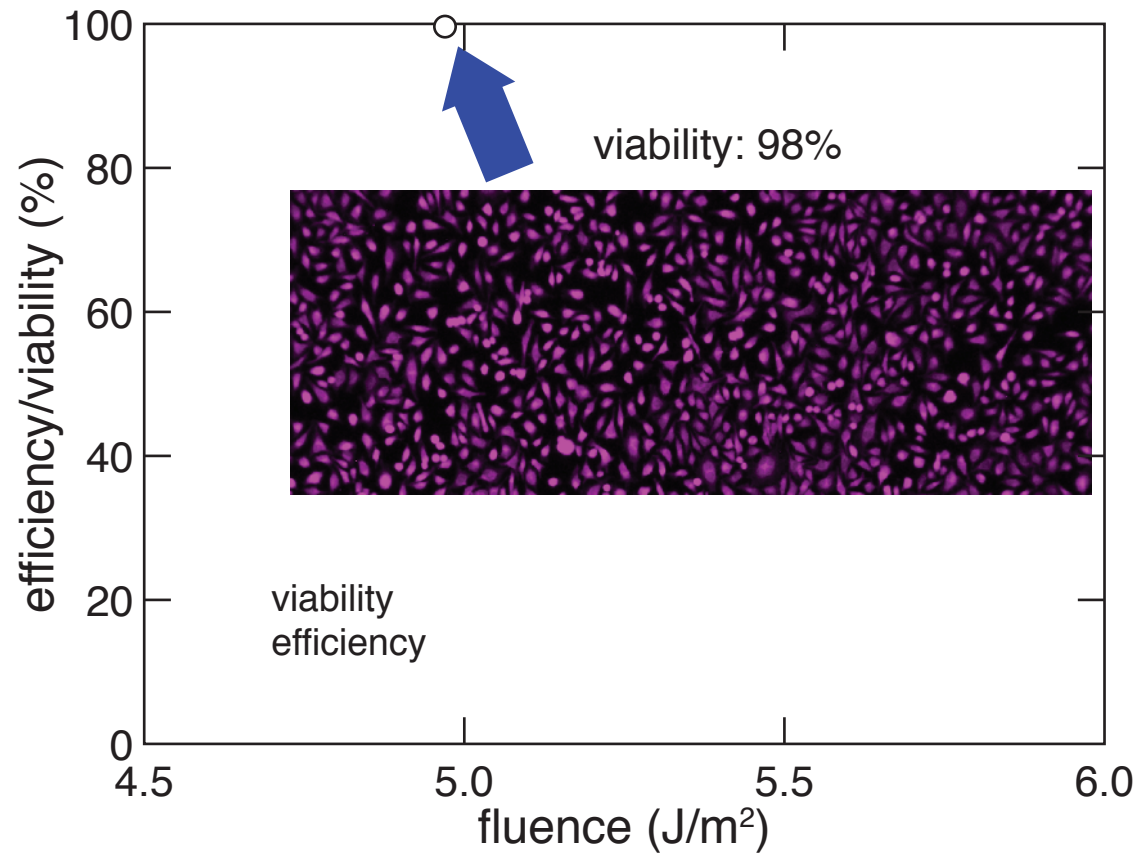
98% of cells

viability

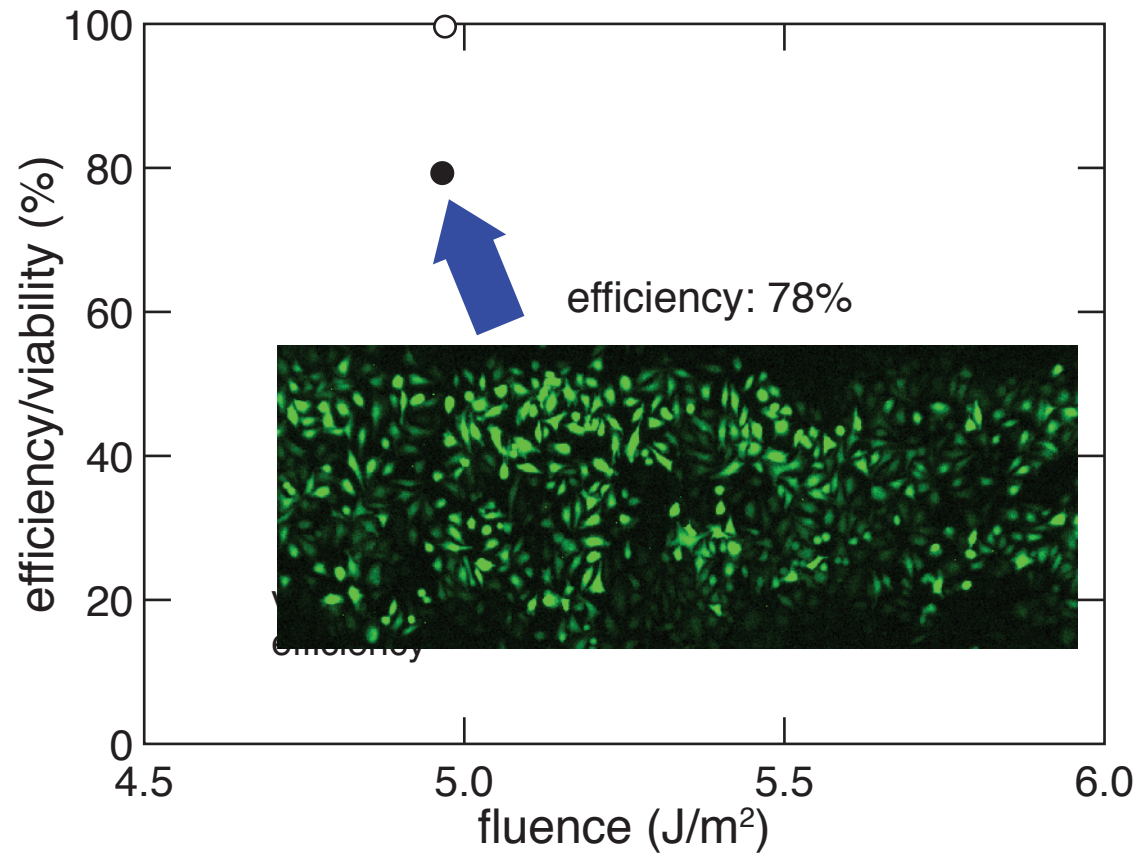
optimizing fluence



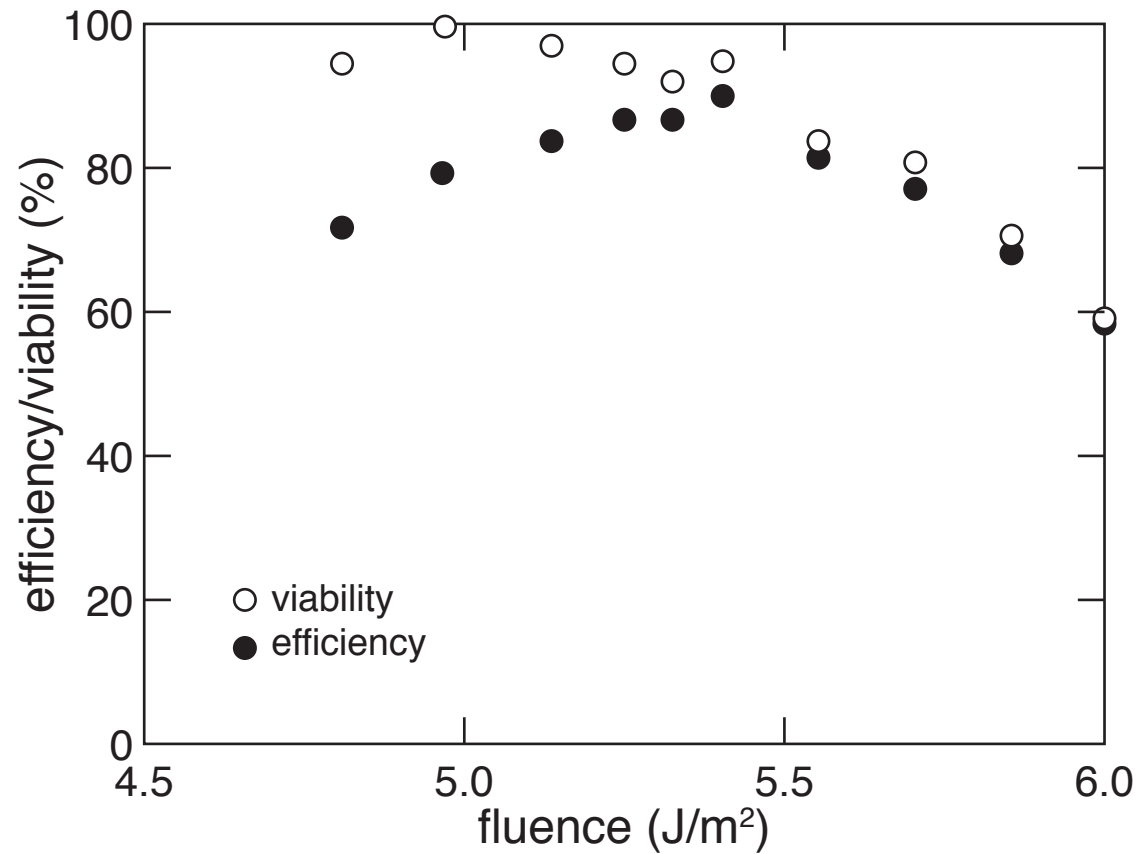
optimizing fluence



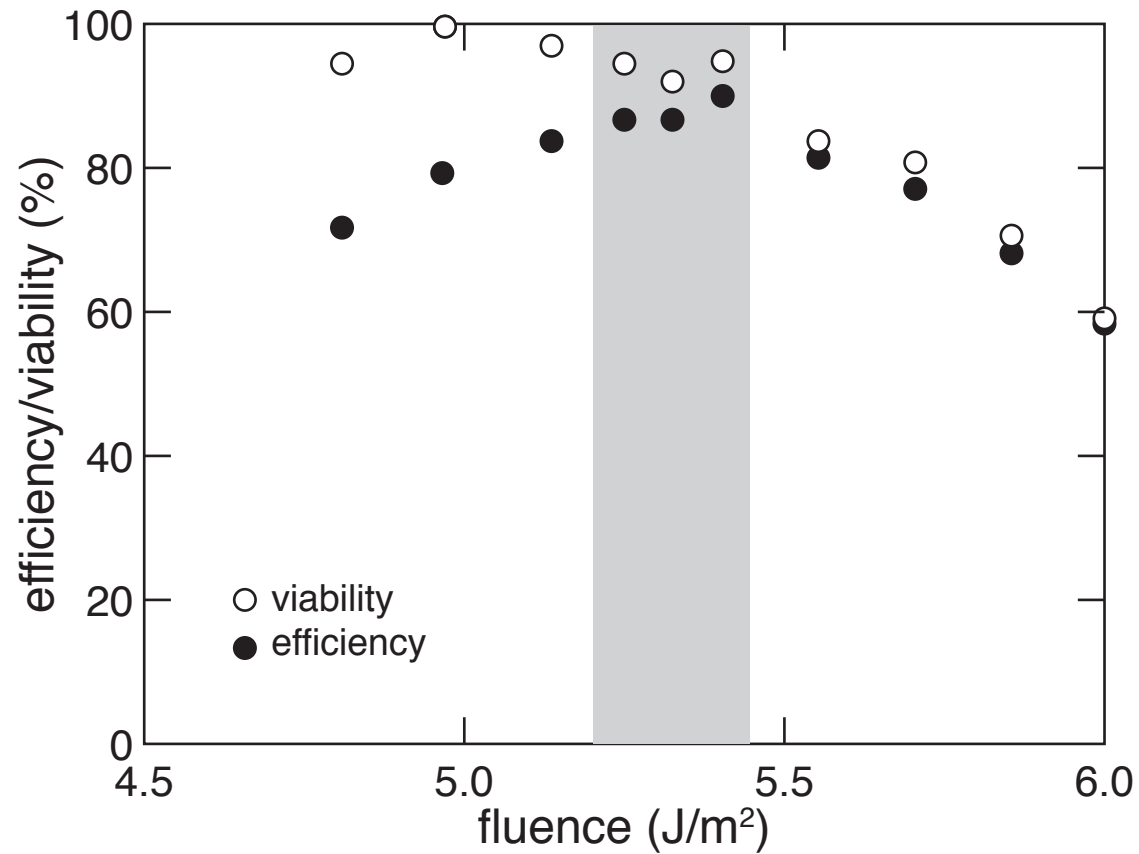
optimizing fluence



optimizing fluence



optimizing fluence



goal

Viability	Efficiency	Throughput	Versatility
H	H	H	H

1 substrate

2 mechanism

3 results

goal

Viability	Efficiency	Throughput	Versatility
✓	H	H	H

1 substrate

2 mechanism

3 results

goal

Viability	Efficiency	Throughput	Versatility
✓	✓	H	H

1 substrate

2 mechanism

3 results

The background of the slide is a grayscale micrograph showing a dense layer of cells growing on a substrate with a repeating pattern of small, upward-pointing triangles. The cells are concentrated in the valleys between these triangles.

throughput

400 mm² in 2 s \approx 10⁶ cells/min!

1 substrate

2 mechanism

3 results

goal

Viability	Efficiency	Throughput	Versatility
✓	✓	H	H

1 substrate

2 mechanism

3 results

goal

Viability	Efficiency	Throughput	Versatility
✓	✓	✓	H

1 substrate

2 mechanism

3 results



versatility

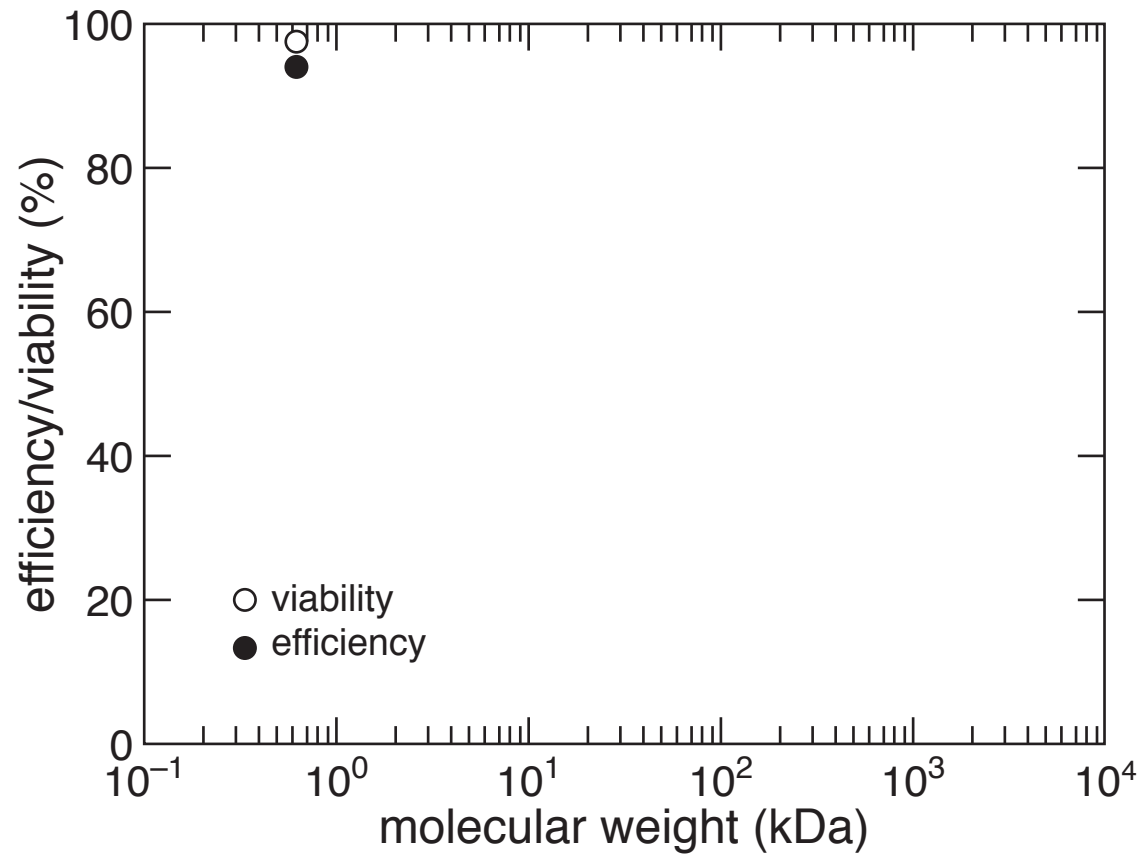
cargo size, cell type

1 substrate

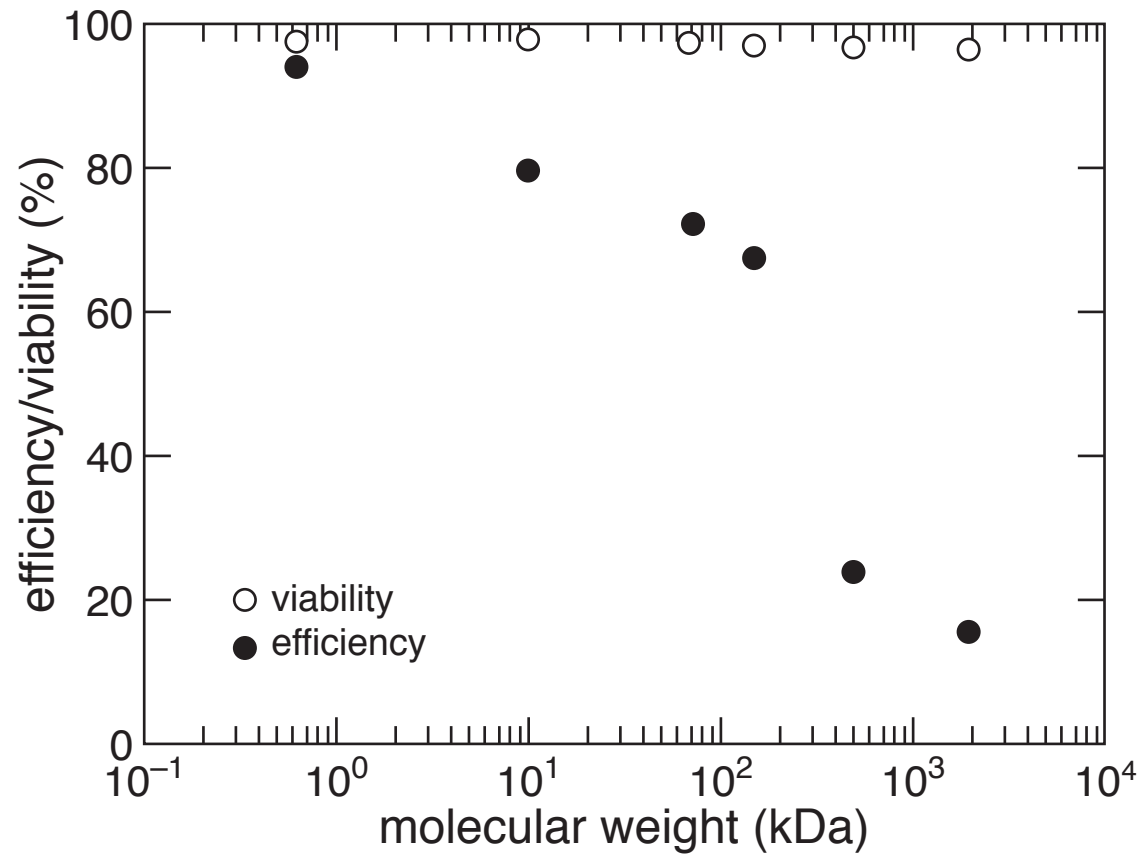
2 mechanism

3 results

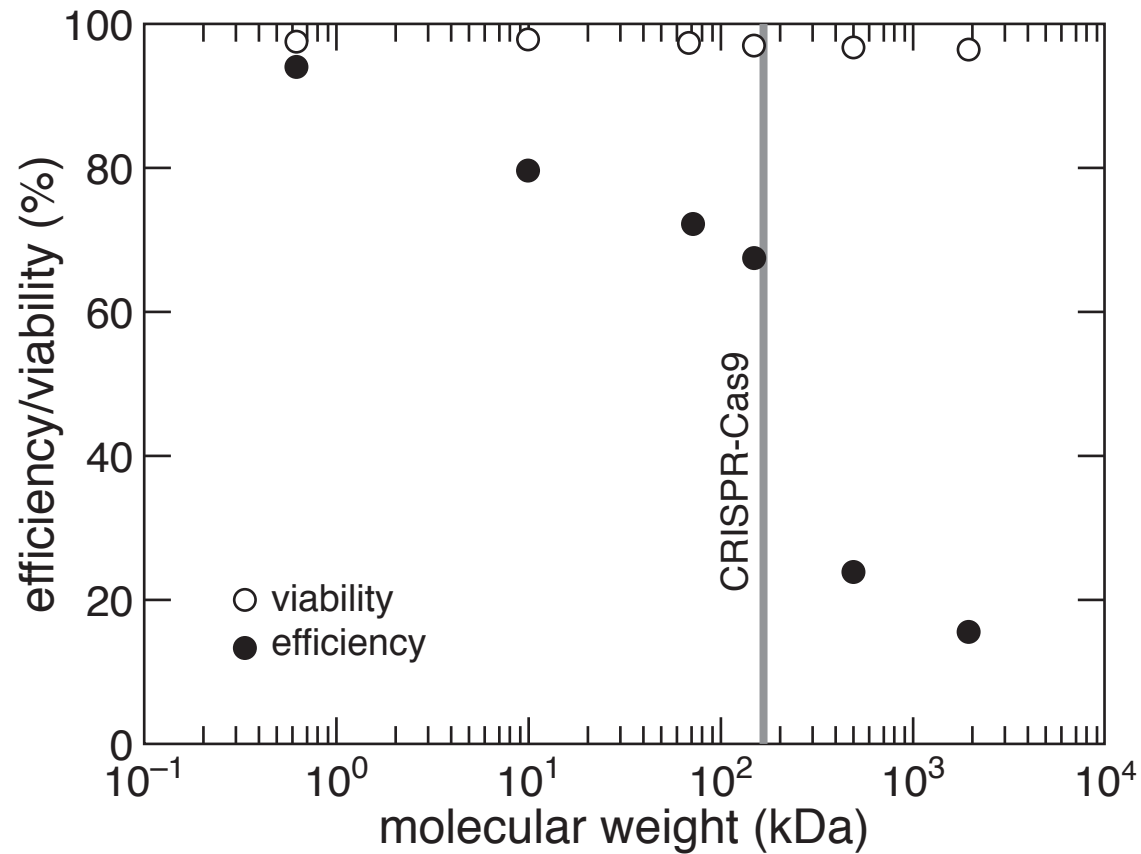
cargo-size



cargo-size

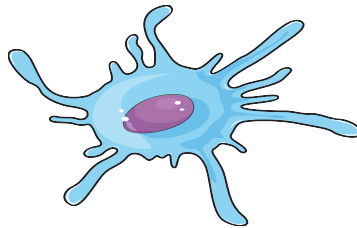


cargo-size

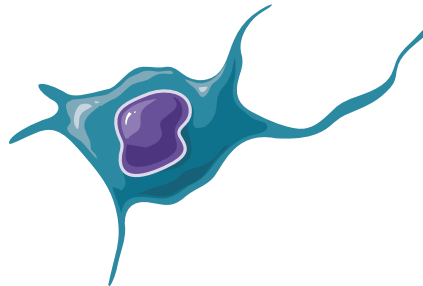


cell type

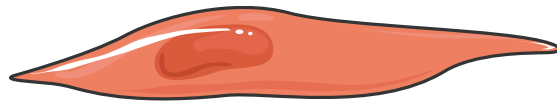
adherent



HeLa



breast



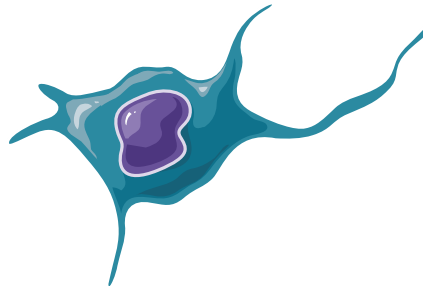
muscle

cell type

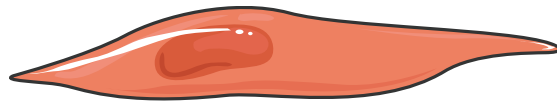
adherent



HeLa



breast



muscle

cell type

adherent



HeLa



breast



muscle

cell type

adherent



HeLa



breast



muscle

cell type

adherent



HeLa

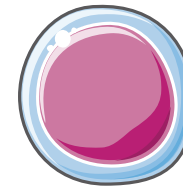


breast

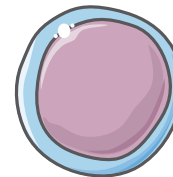


muscle

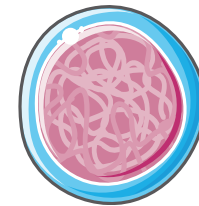
suspension



bone marrow



immune



stem

cell type

adherent



HeLa



breast

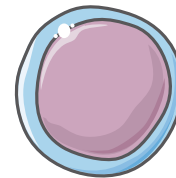


muscle

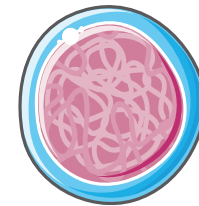
suspension



bone marrow



immune



stem

goal

Viability	Efficiency	Throughput	Versatility
✓	✓	✓	H

1 substrate

2 mechanism

3 results

goal

Viability	Efficiency	Throughput	Versatility
✓	✓	✓	✓

1 substrate

2 mechanism

3 results



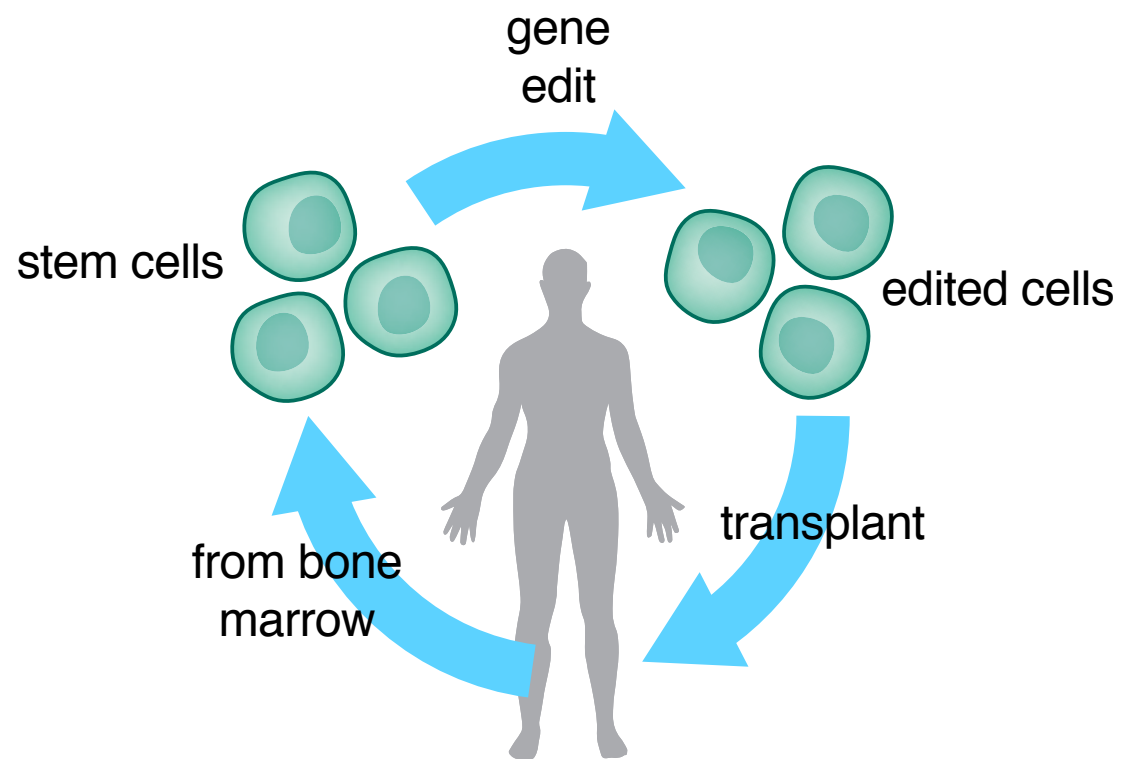
**can deliver CRISPR-Cas9-sized
molecules to suspension cells**

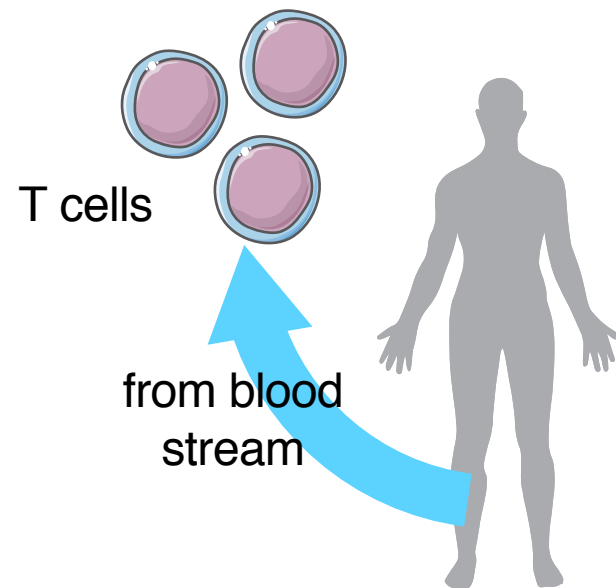
1 substrate

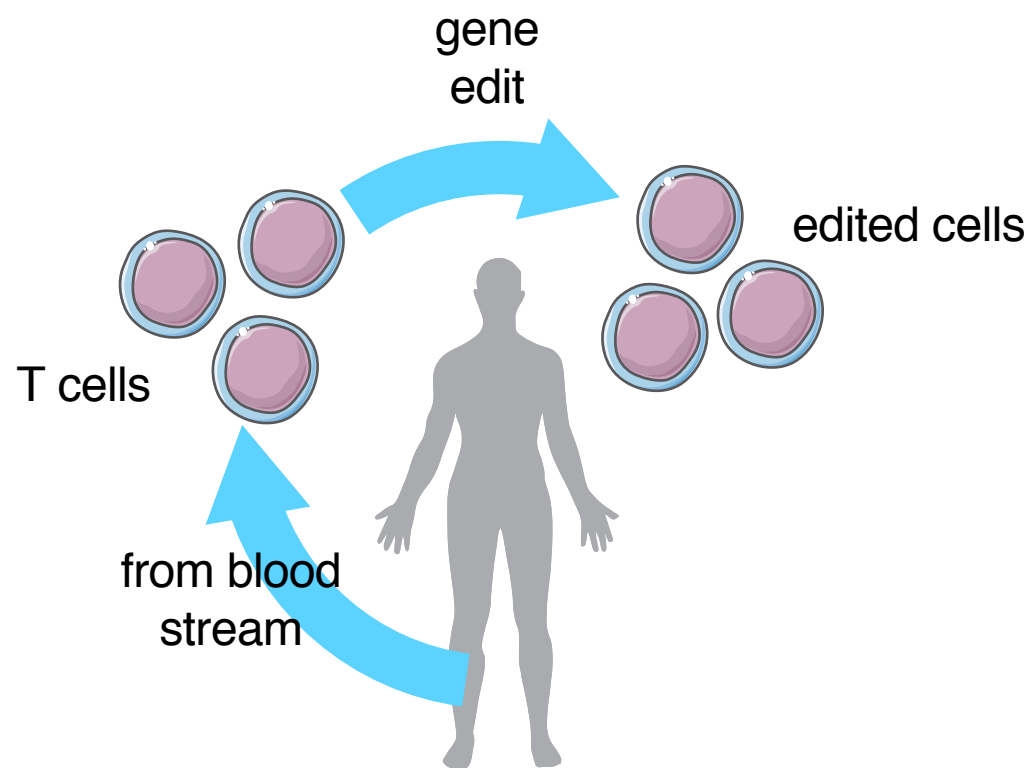
2 mechanism

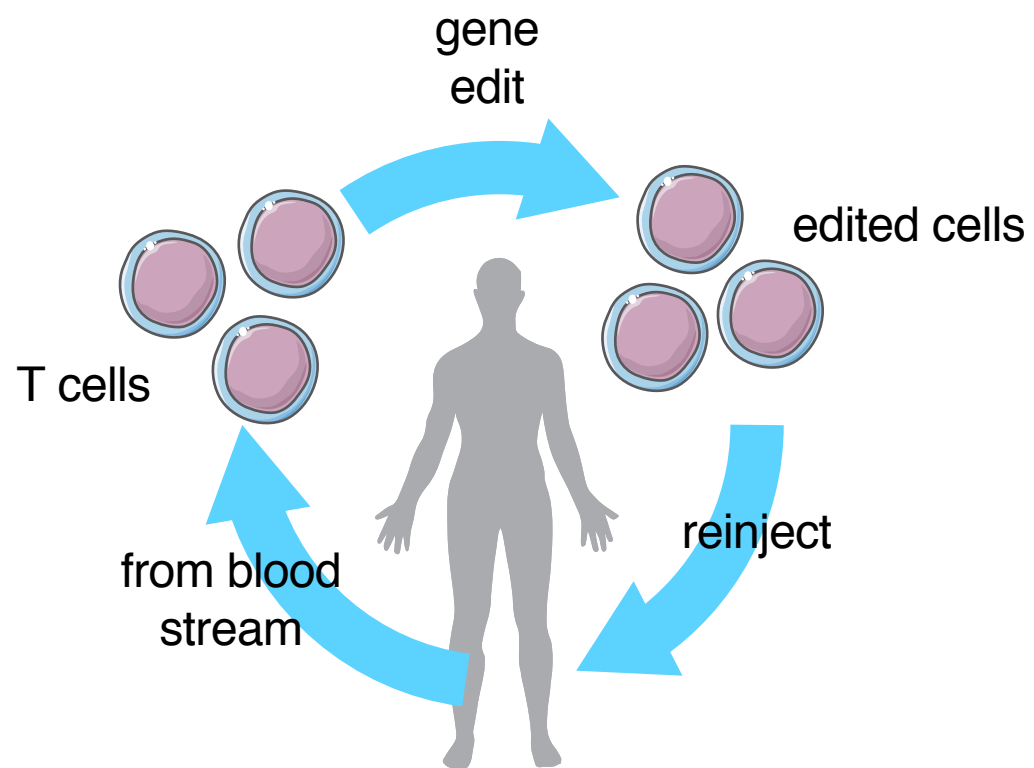
3 results

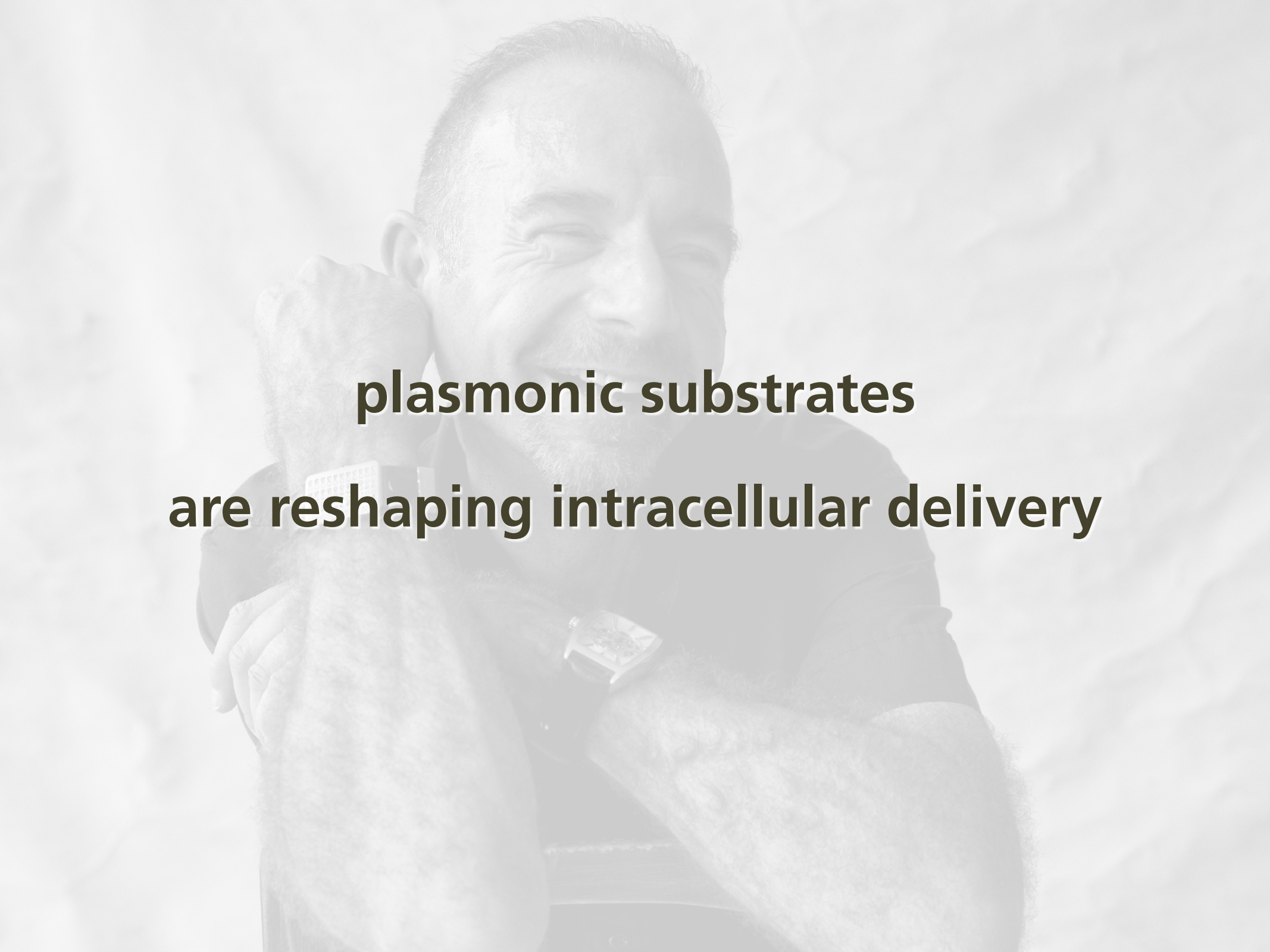












**plasmonic substrates
are reshaping intracellular delivery**

**Eric Diebold, Alex Heisterkamp, Valeria Nuzzo, Sebastien Courvoisier,
Jean-Pierre Wolf, Jun Chen, Marinus Huber, Michel Meunier, Daryl
Vulis, Alex Raun, Weilu Shen, Jeffery Nelson, Rahul Palchaudhuri**

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