Plasmonic cell transfection using micropyramids

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MAZUR GROUP

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HARVARD UNIVERSITY

[1] UNIVERSITY OF GENEVA, SWITZERLAND

Outline

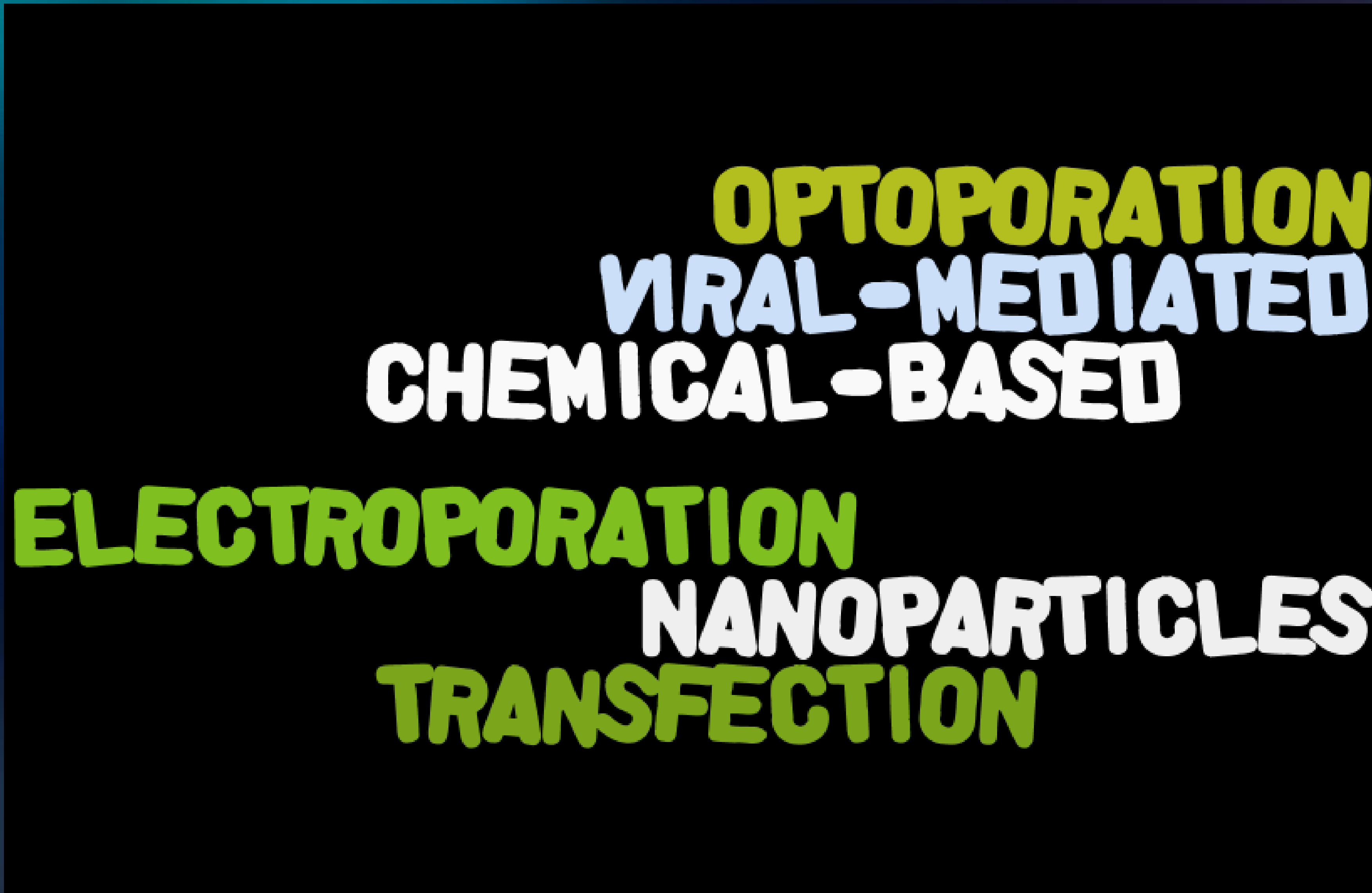
Motivation

Design and Fabrication

Cavitation Bubbles

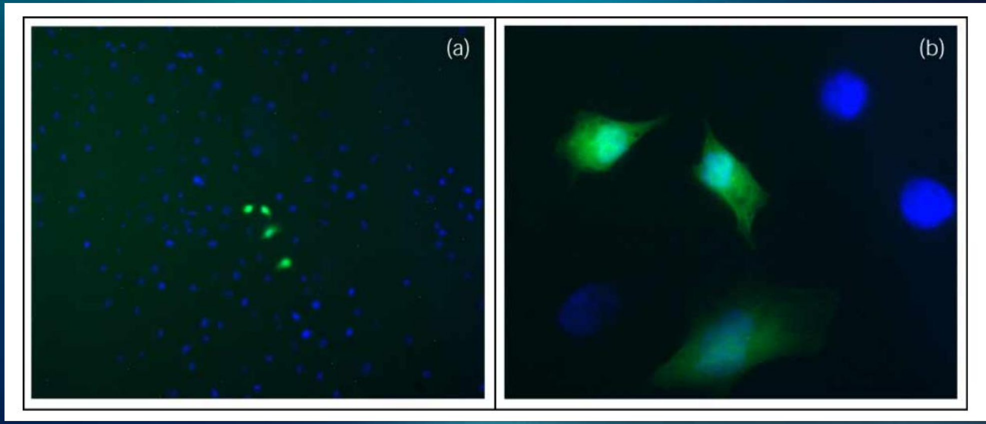
Poration

Wordle.com, Wikipedia.com

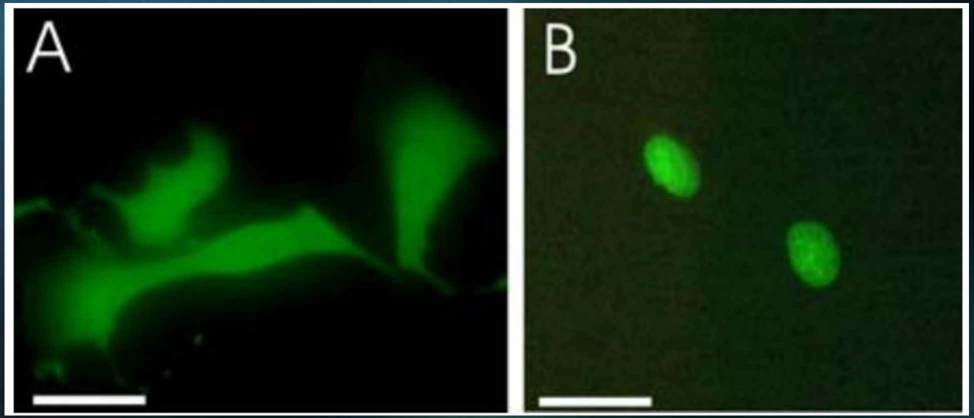

OPTOPORATION
VIRAL-MEDIATED
CHEMICAL-BASED
ELECTROPORATION
NANOPARTICLES
TRANSFECTION

Wordle.com

Optotransfection has many benefits



D. Stevenson, B. Agate, et al. OPTICS EXPRESS 2006.



J. Baumgart et al. Journal of Laser Micro/Nanoengineering Vol. 4, No. 2, 2009

Can we scale-up?

Can we retain the benefits of optotransfection, while targeting many more cells at once?

Surface plasmons for transfection

Can we retain the benefits of optotransfection, while targeting many more cells at once?

… by manipulating the effects of ***localized surface plasmon resonances (LSPRs)***

Surface plasmons for transfection

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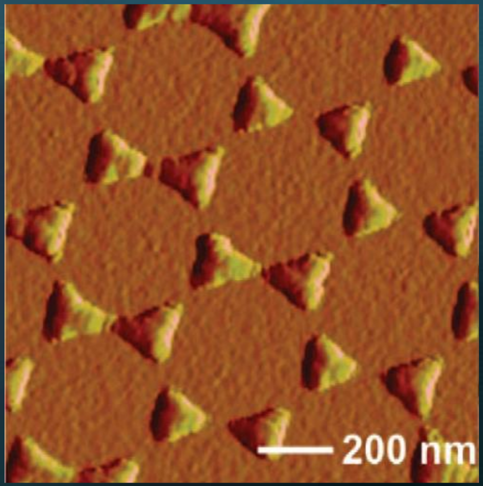
Form 'hotspots' of evanescent enhancement

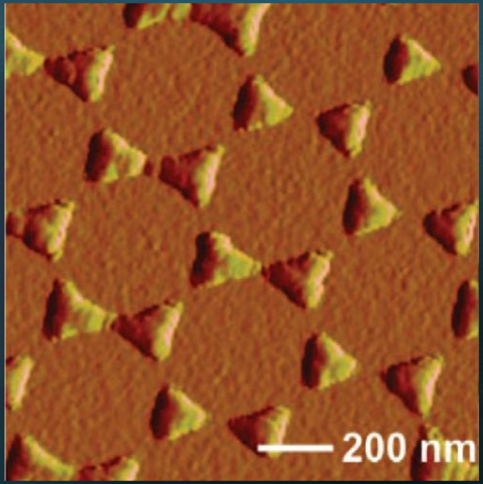


Camden et al.,
Accounts of Chemical Research 2008

collective electron density oscillations found in noble metal nanostructures

Surface plasmons for transfection

Goal: to transfect 100,000 cells per minute using a plasmonic susbtrate



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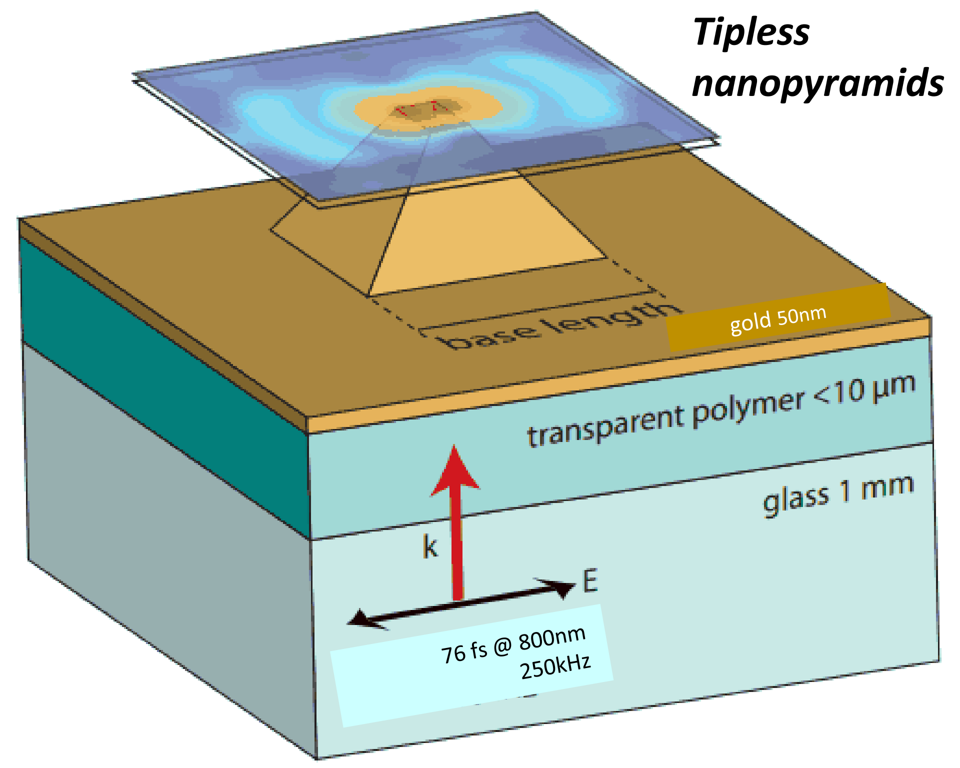
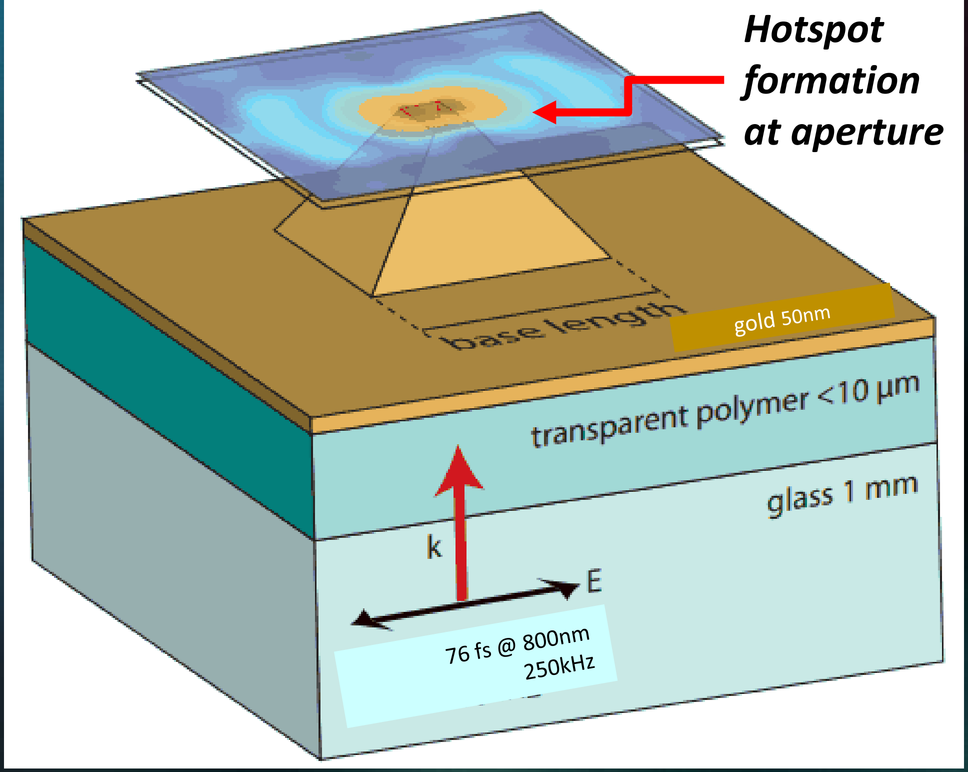
Substrate Design

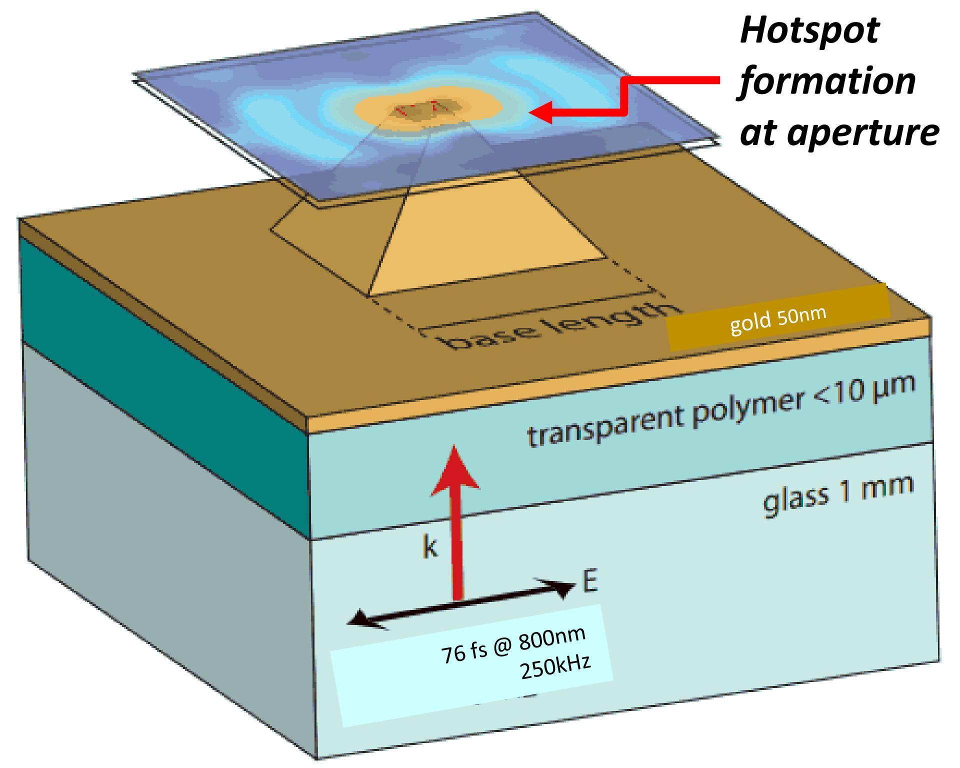


Substrate Design

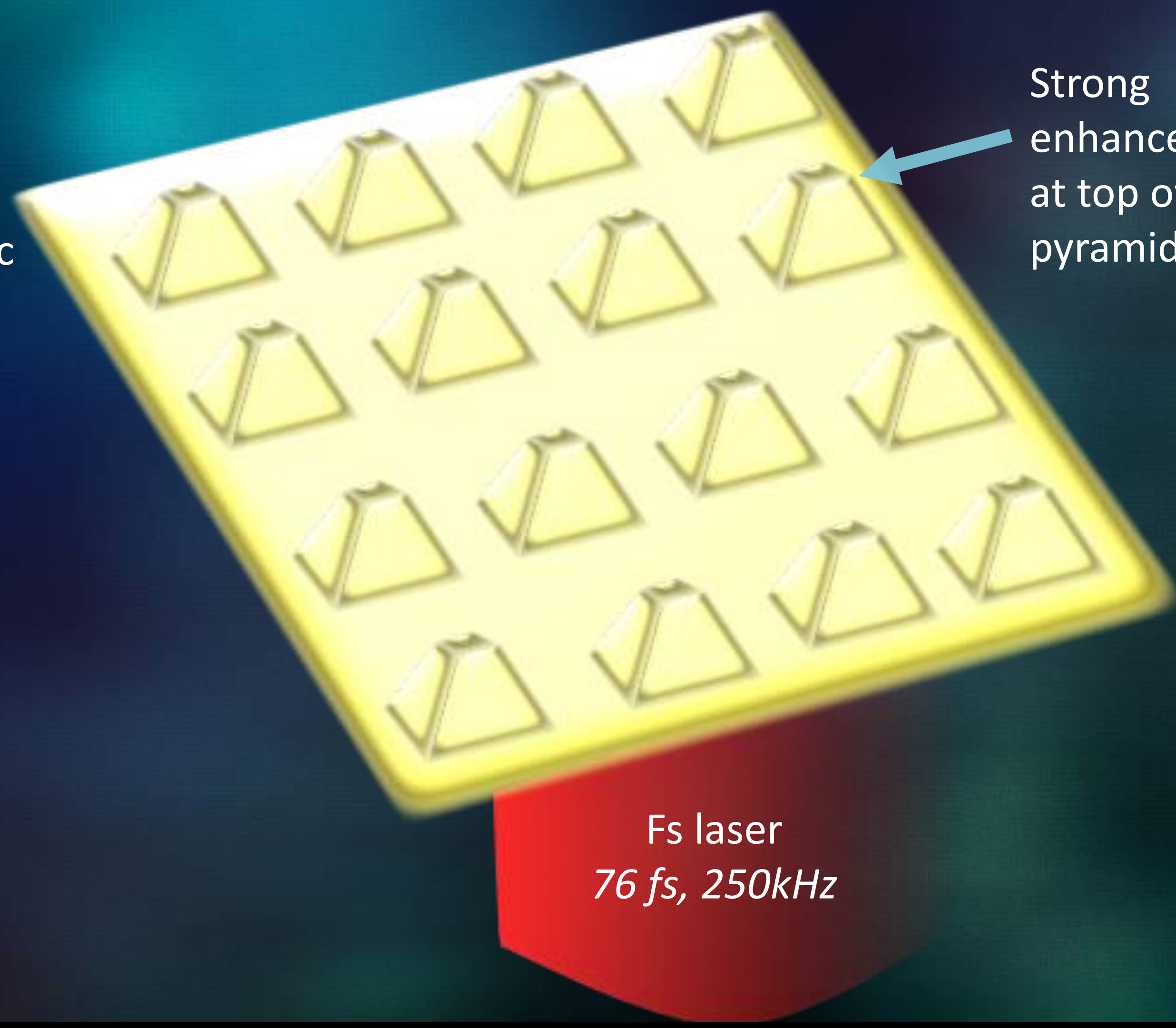


Substrate Design

- *High near-field enhancement*
- *Hot spot near membrane*
- *High biocompatibility*
- *Unobstructed area for poration*



Experimental Design



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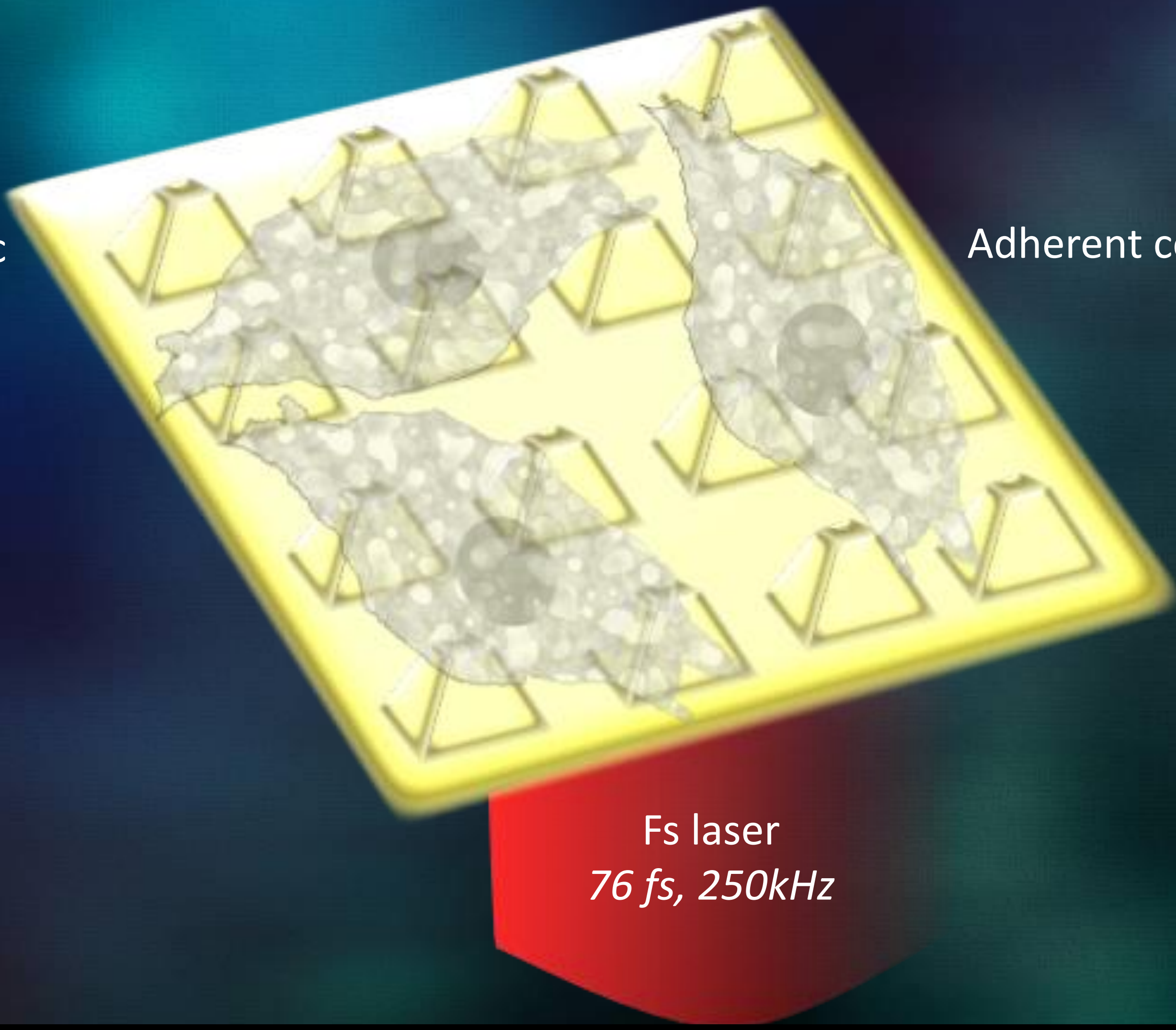
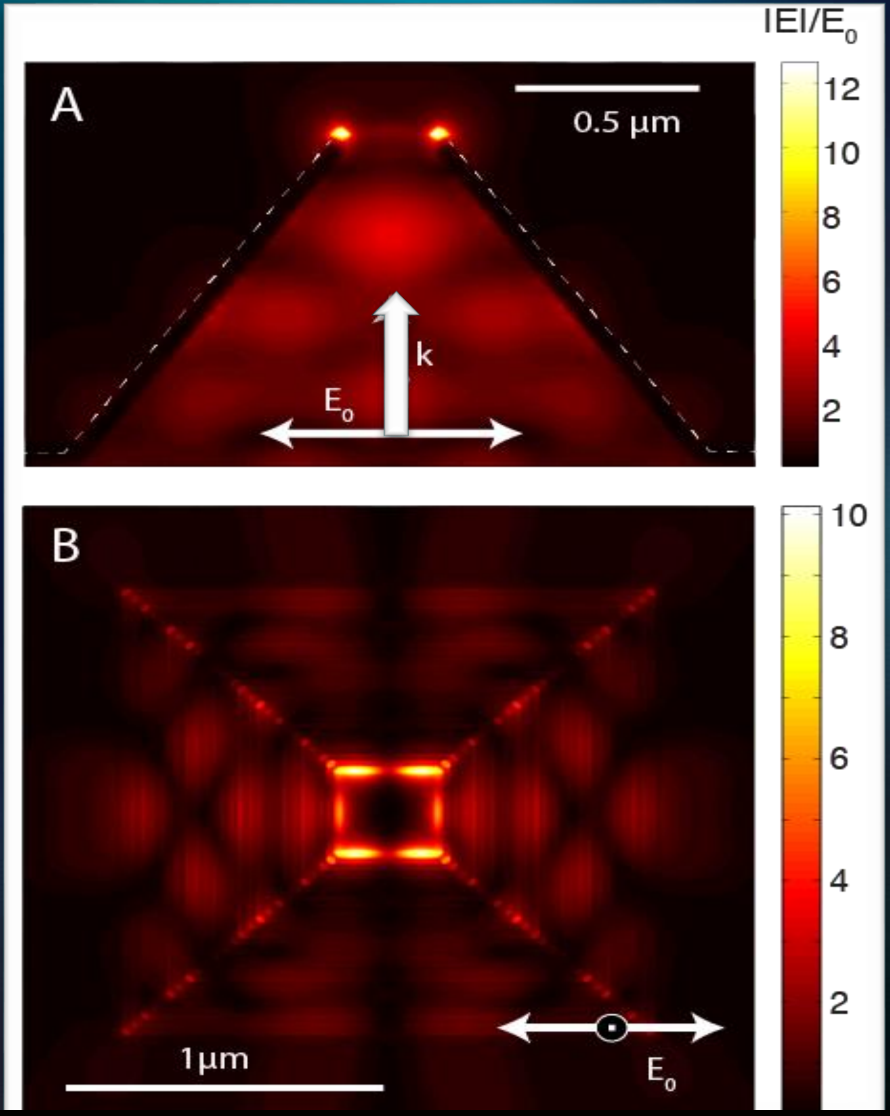


Simulations to optimize parameters



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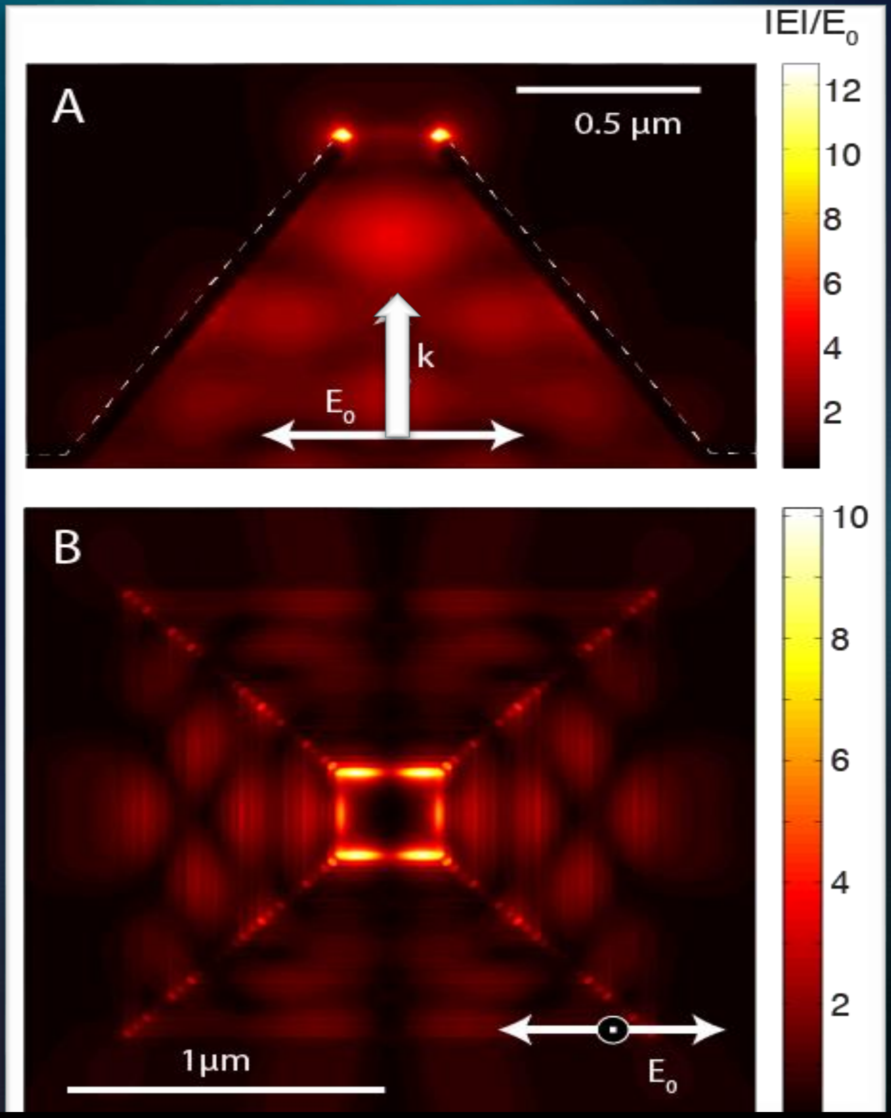


1 *Aperture Size*

2 *Gold Thickness*

3 *Base Length*

4 *Separation*

High-precision fabrication

Harvard University
Center for Nanoscale Systems

High-precision fabrication

Harvard University
Center for
Nanoscale
Systems

High-precision fabrication

Harvard University
Center for Nanoscale Systems

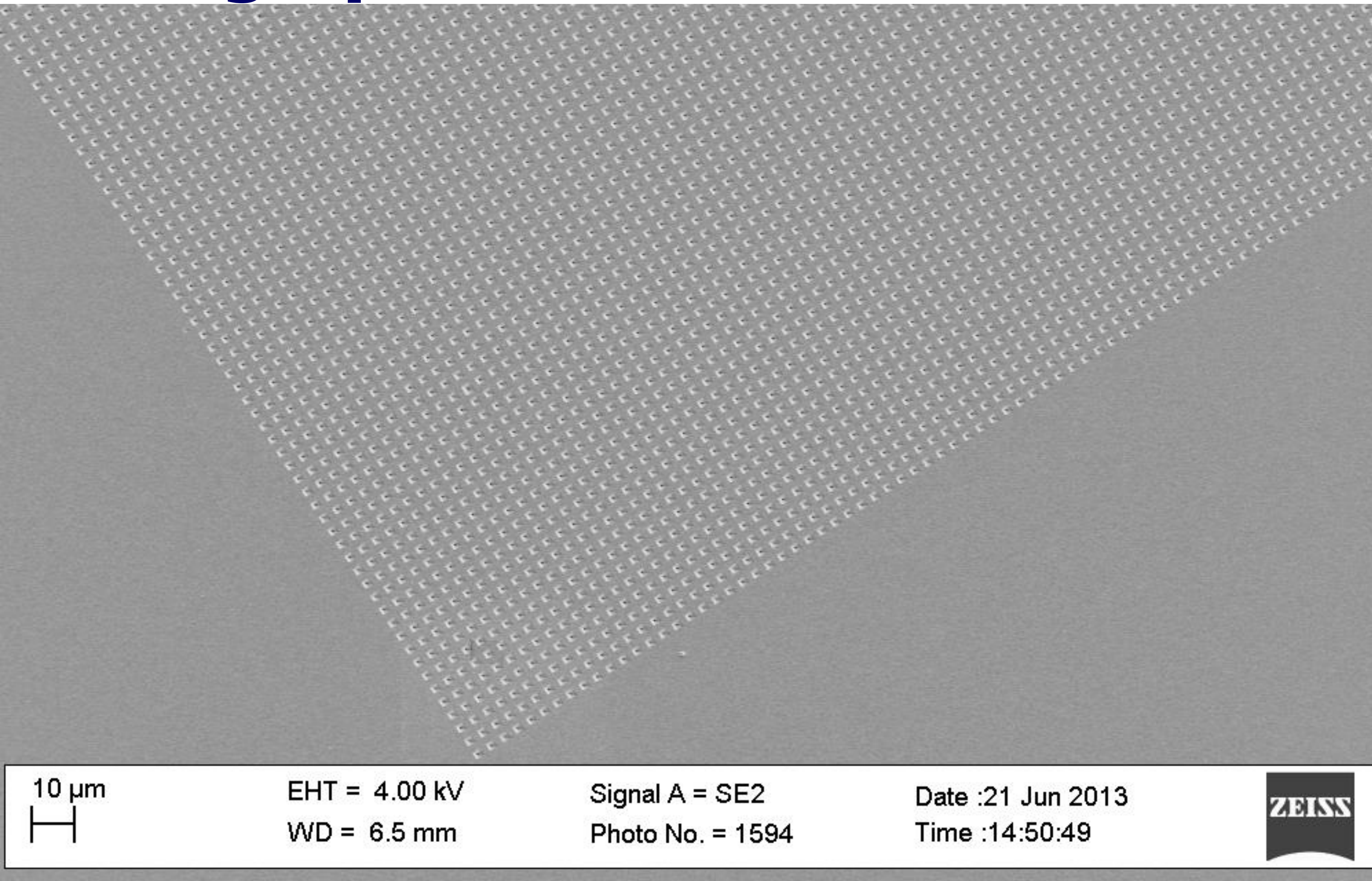
E-Beam lithography

Anisotropic etching

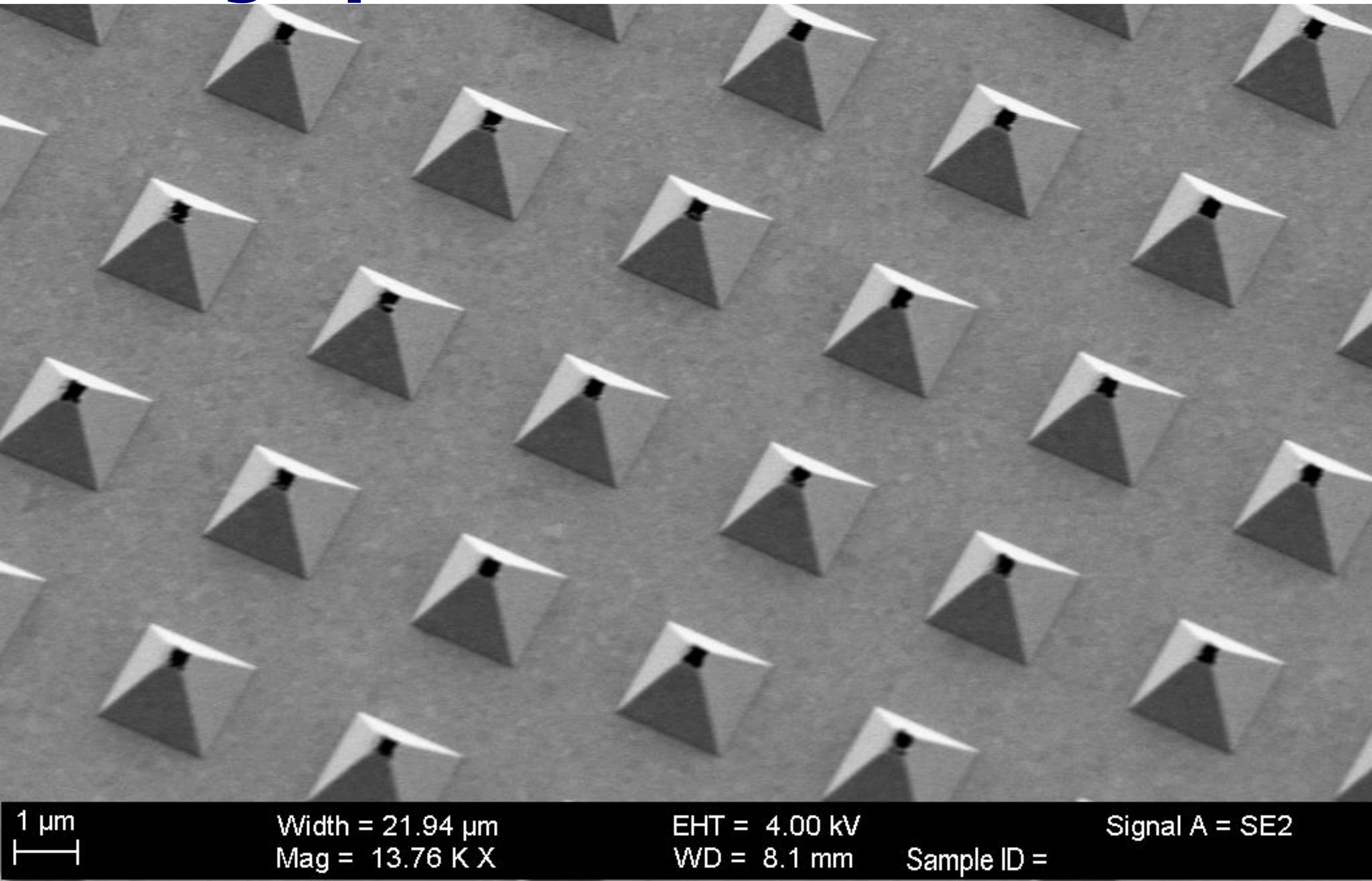
Gold deposition

Template-stripping

High-precision nanostructures



High-precision nanostructures



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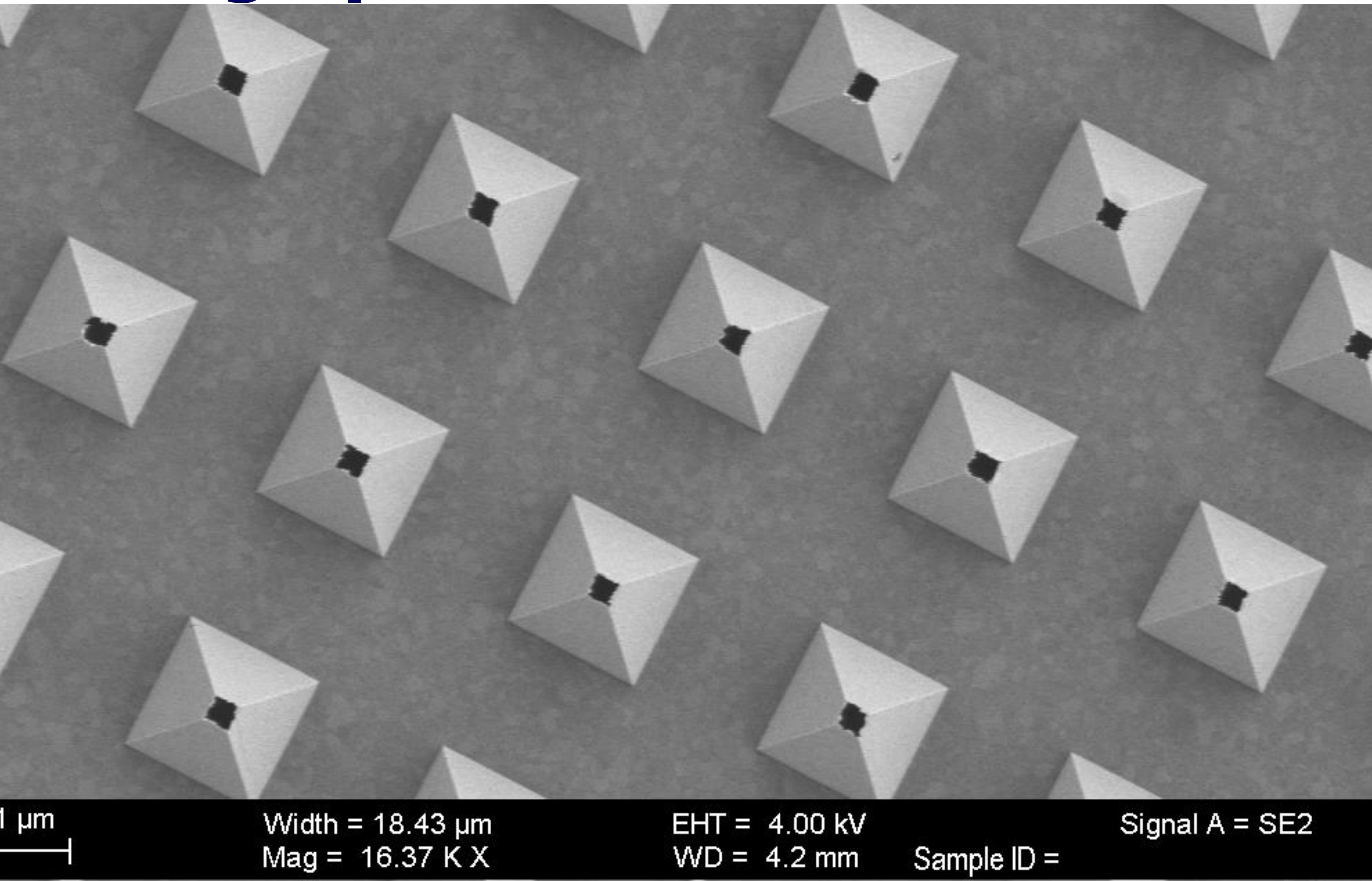

Characterization of nanostructures

Surface-Enhanced Raman Spectroscopy

Atomic Force Microscopy

Near-field Scanning Optical Microscopy

Second Harmonic Generation

2-Photon Fluorescence

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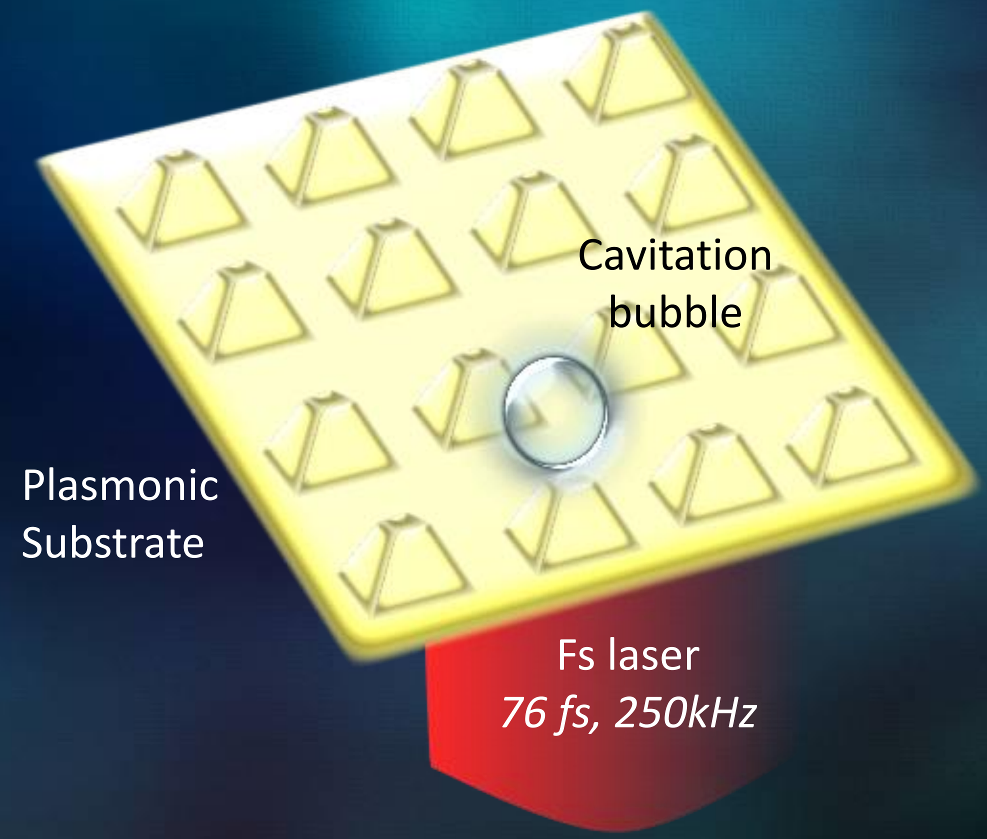
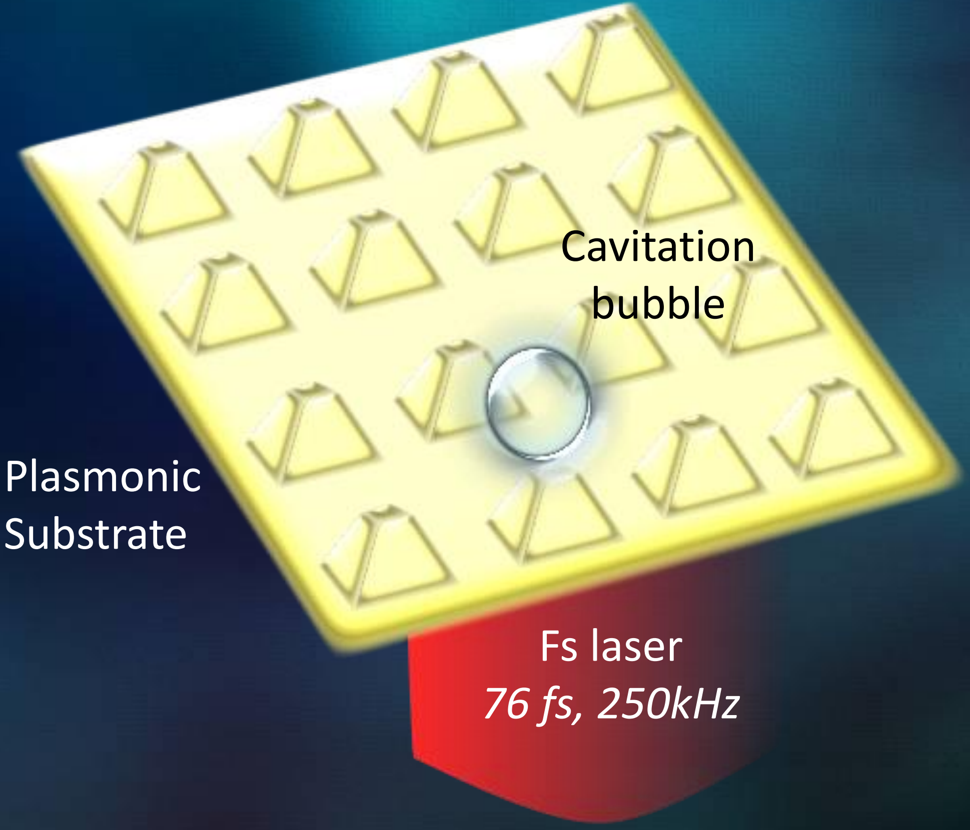
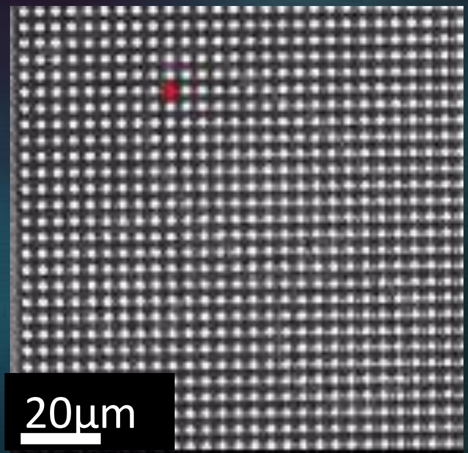
Cavitation bubbles



Cavitation bubbles

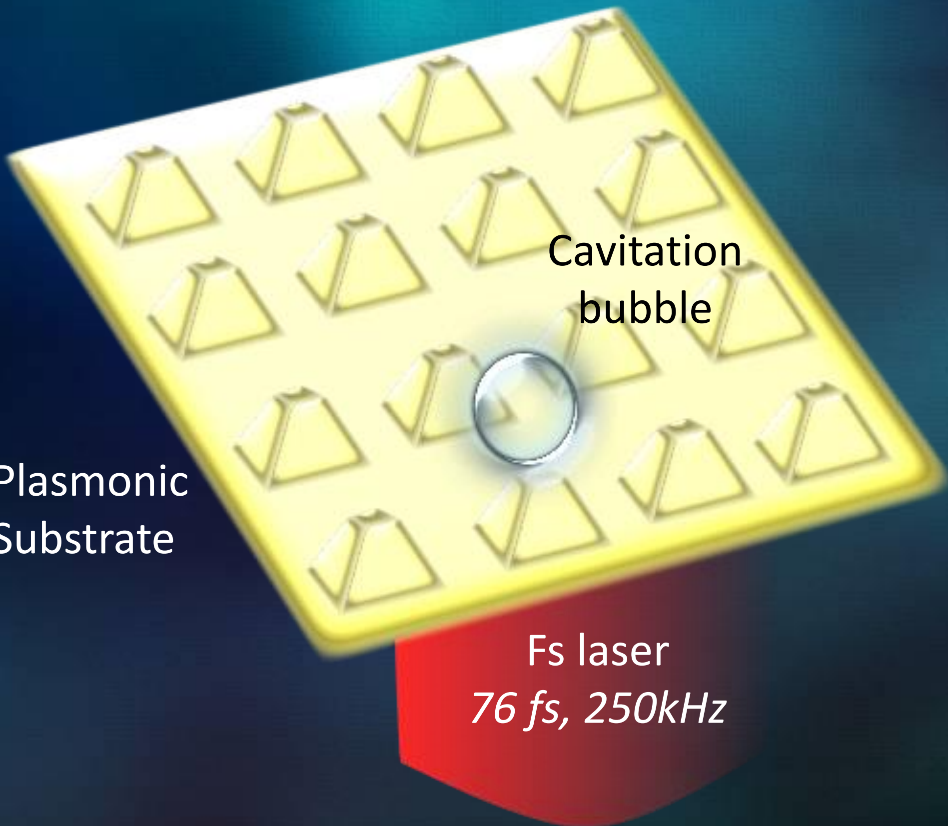




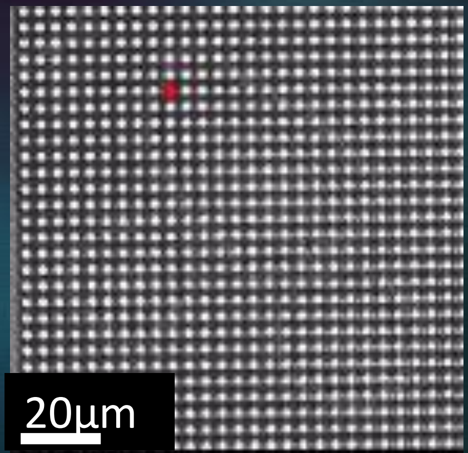
Microbubble formation

beam spot ~ 5µm^2

Cavitation bubbles



What happens to micropyramid post-bubble?



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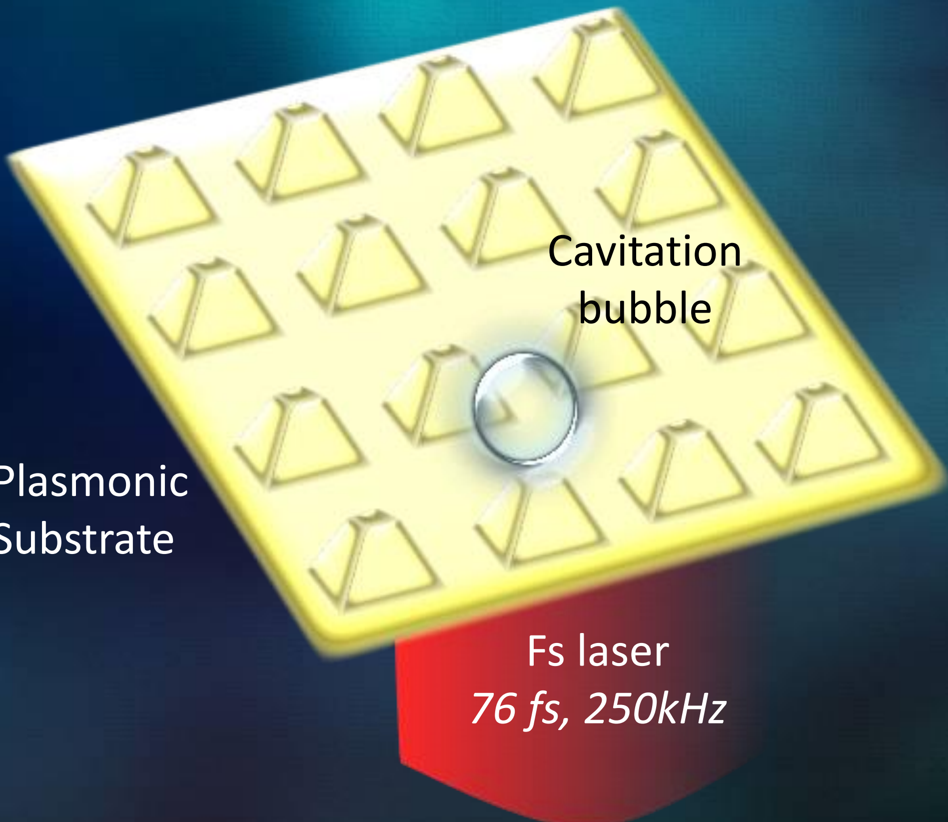

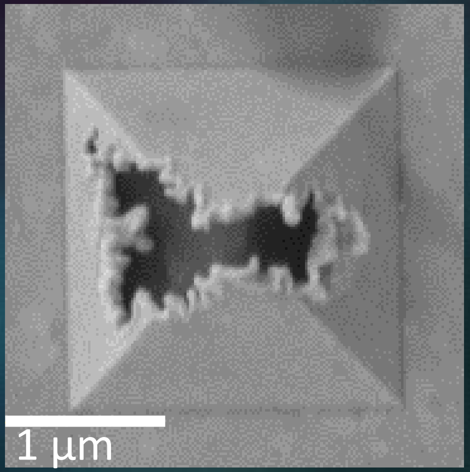

major damage on pyramid

beam spot ~ 5µm^2

Bubble formation damages pyramid

Laser exposure: 1s
Power: 0.25mW
Spot size: 5µm^2
Rep. rate:250 kHz
Pulse duration: 76 fs

2 µm

0.2mW

Power increases

Bubble formation damages pyramid

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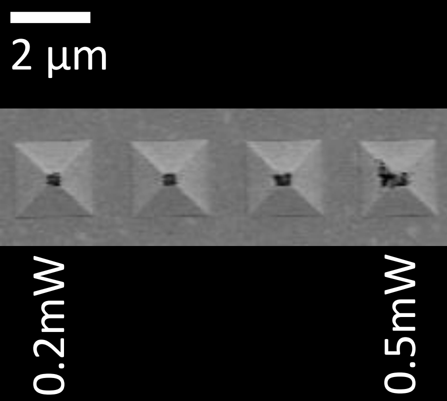
Spot size: 5µm^2
Rep. rate:250 kHz
Pulse duration: 76 fs



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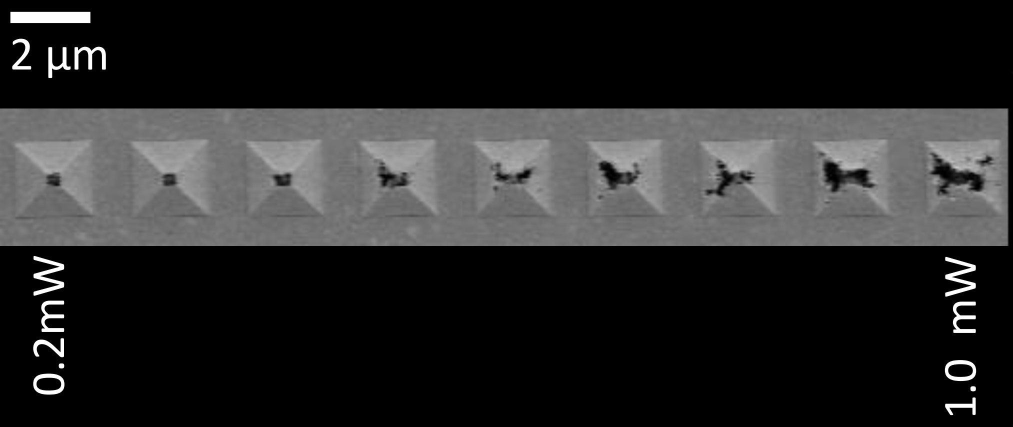

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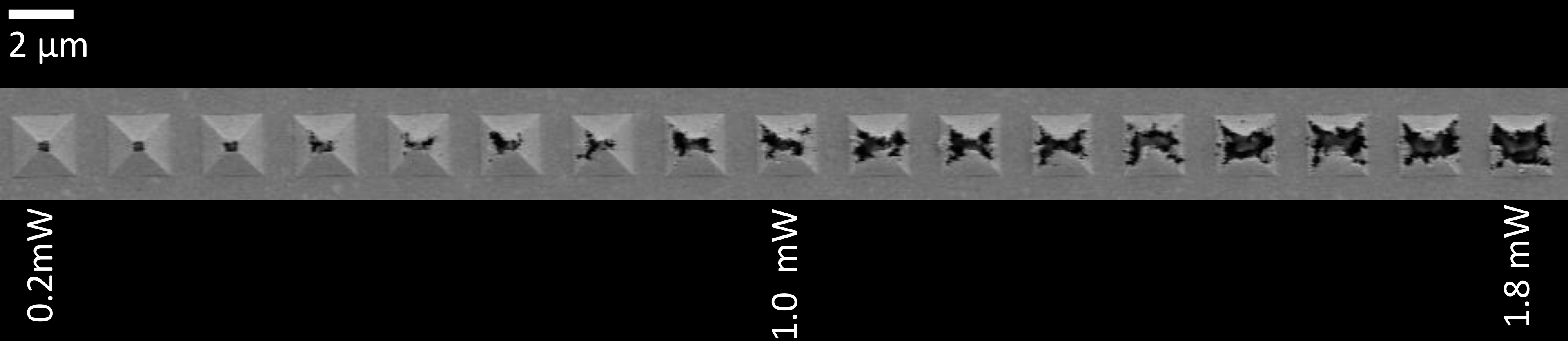

Power increases

Nabiha Saklayen, Mazur Group @ Harvard University

Photonics West 2014

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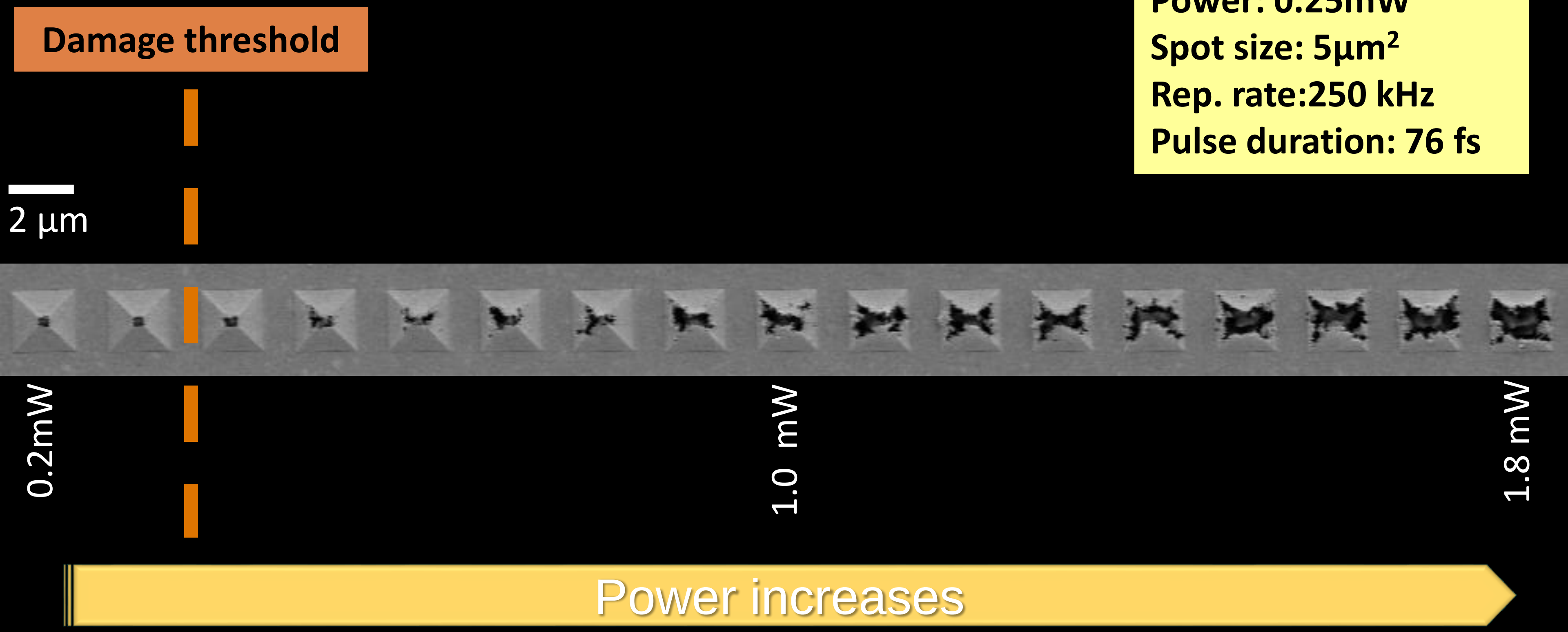

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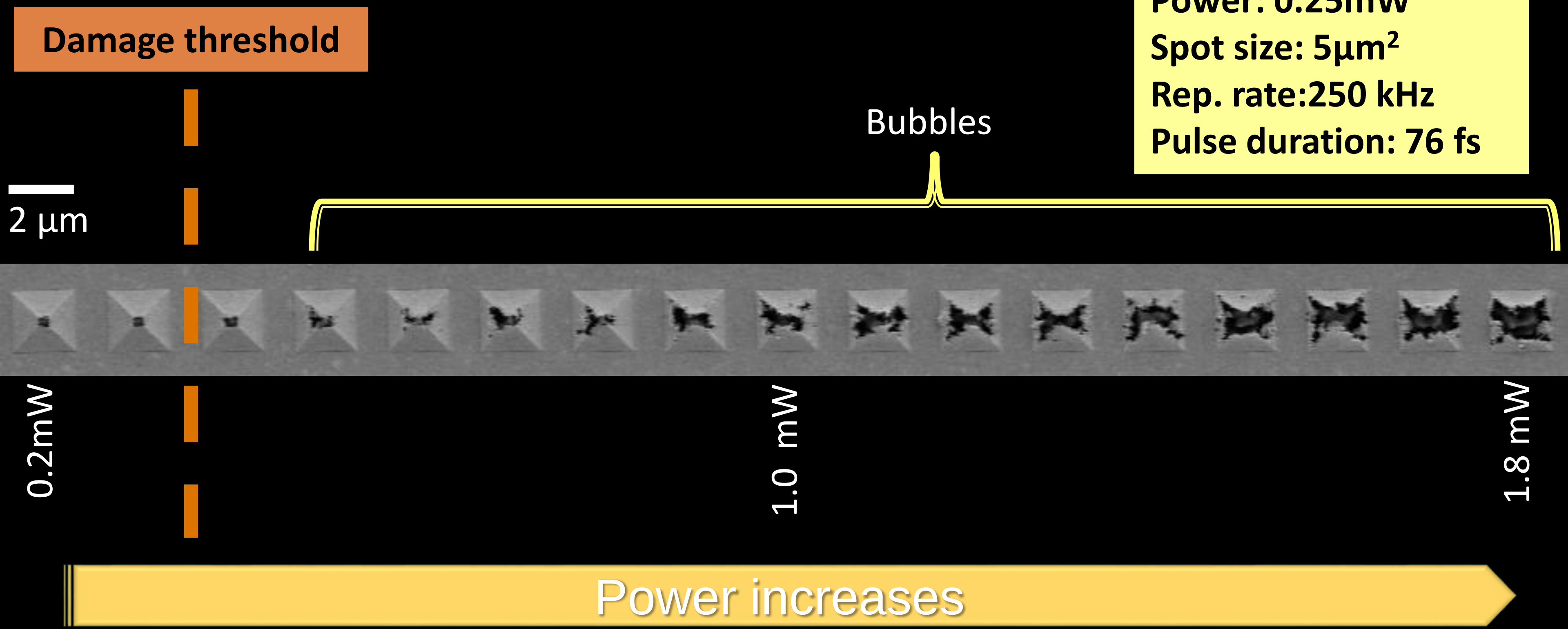

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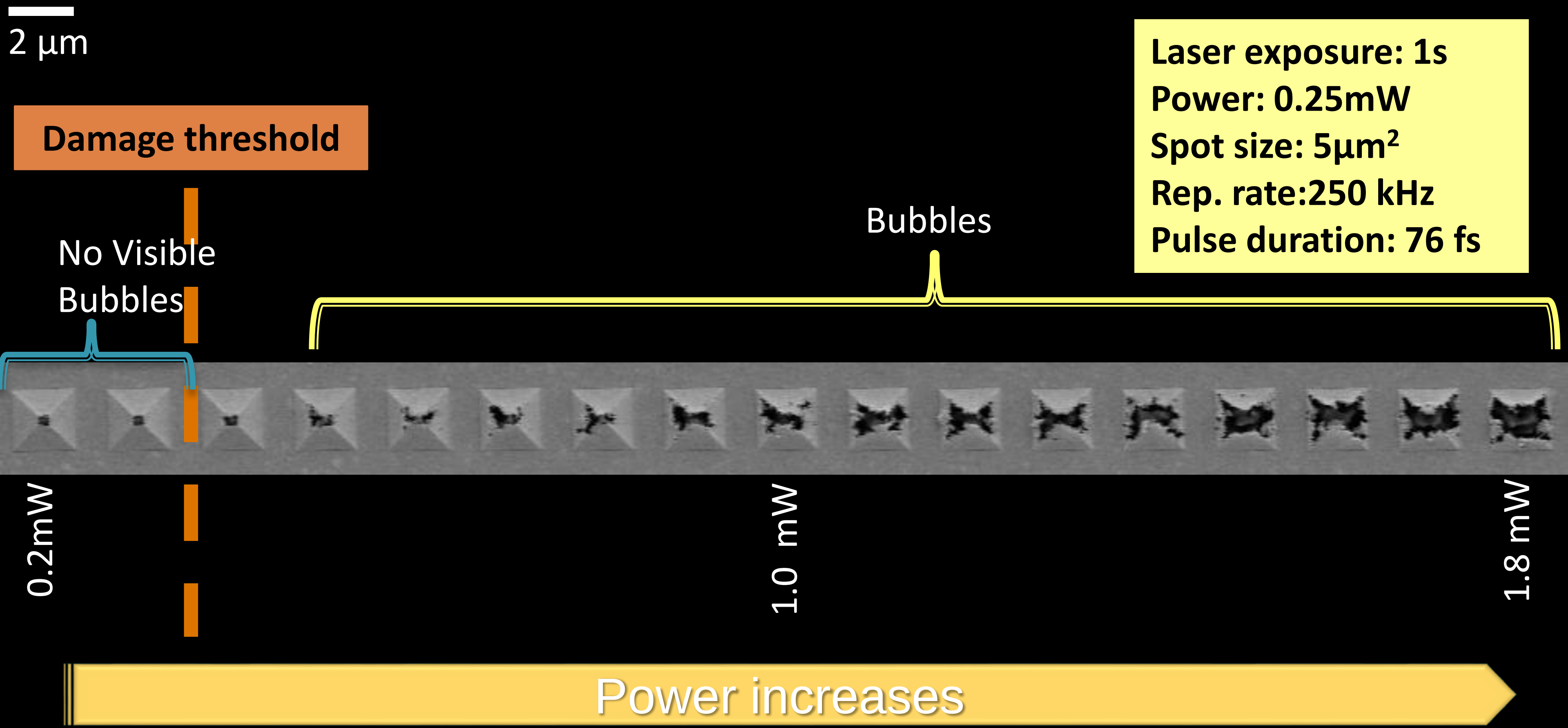
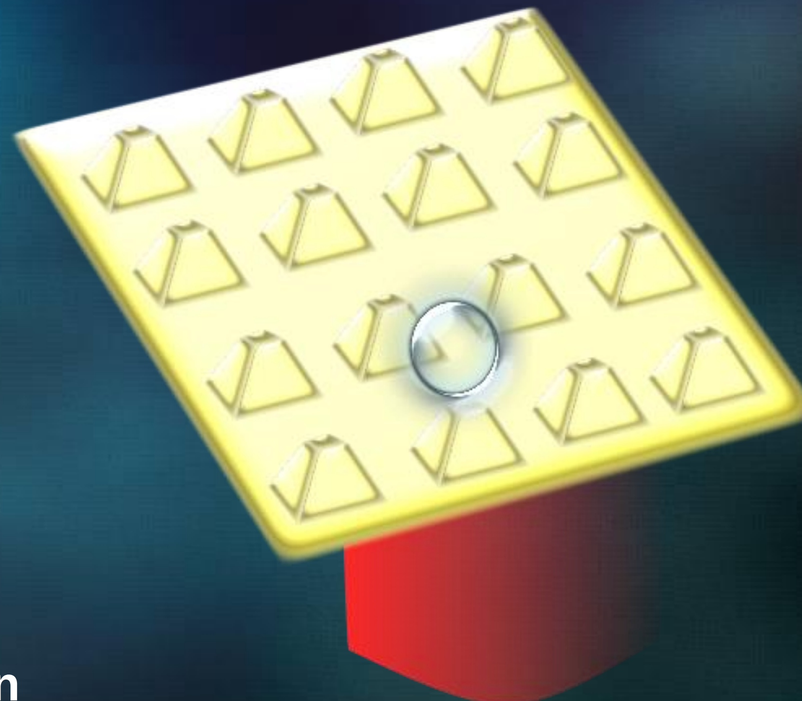


Cavitation bubble dynamics



Session PTue:
JUN CHEN
Optimizing plasmonic transfection using nanostructured substrates
Paper 8972-53
Time: 6:00 PM - 8:00 PM

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Design and Fabrication

Bubble Formation

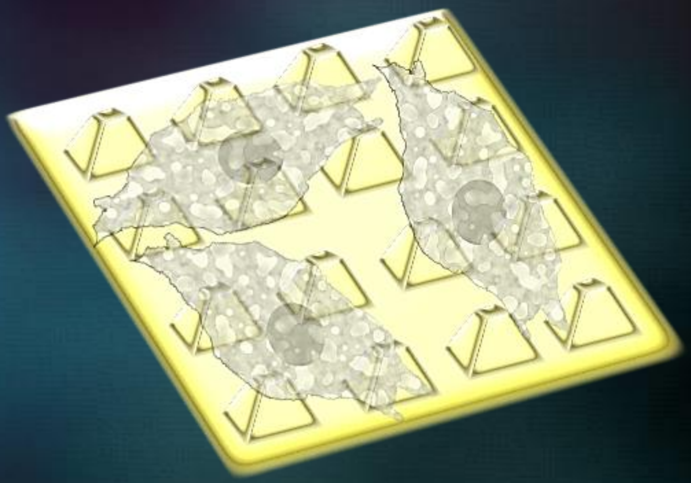
Poration

Experimental Procedure

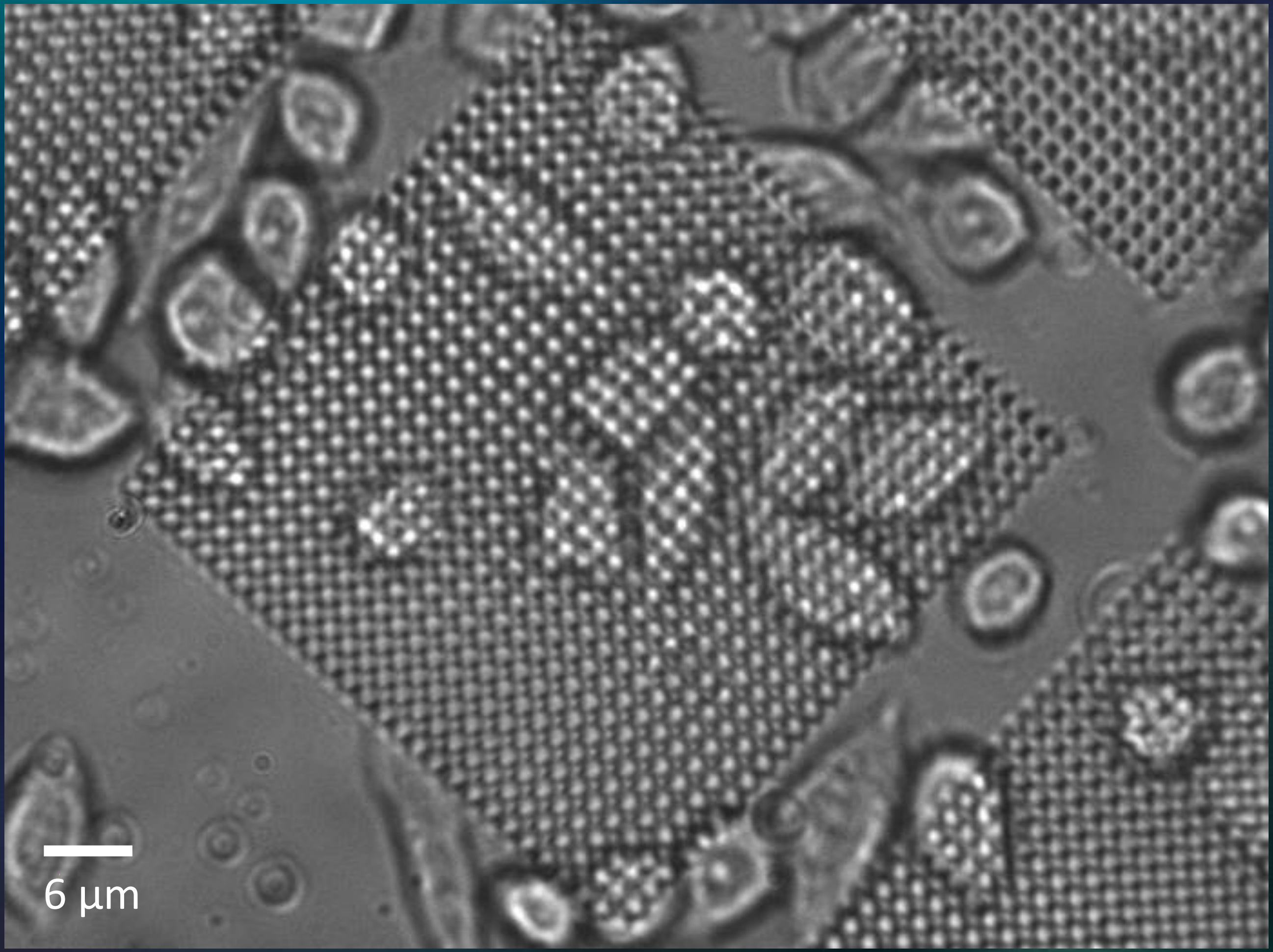
Fabricate Au pyramid substrates

Culture cells on substrates

Porate cells



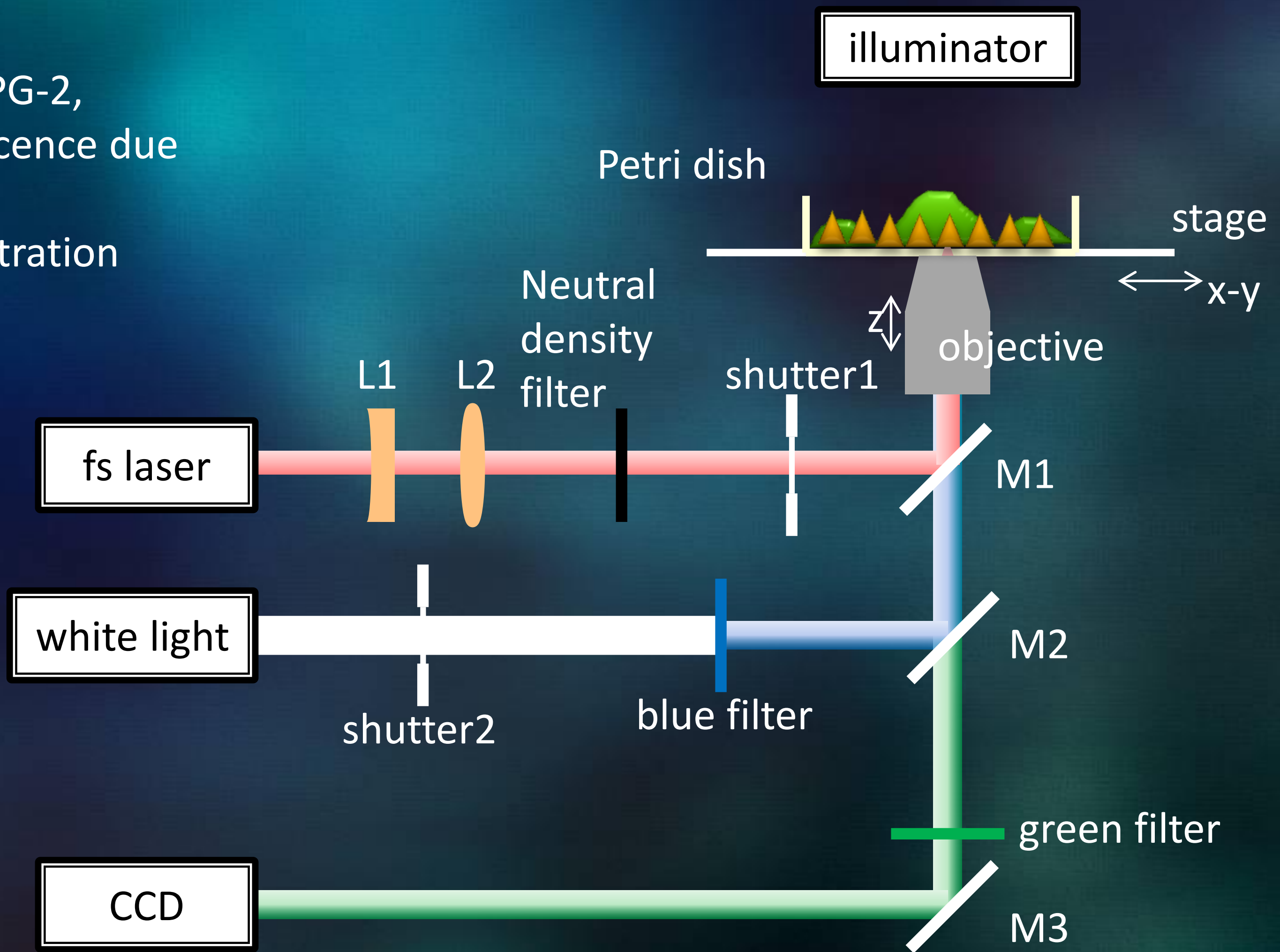
Cell-pyramid adhesion



HeLa S3 cells seeded on pyramid plasmonic substrate

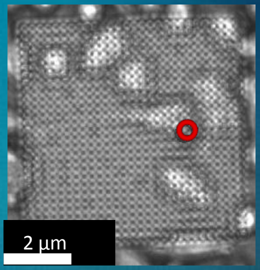
Experimental Setup

Dye: APG-2, fluorescence due to K+ concentration



Microbubble formation in cells

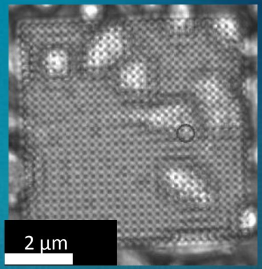
Laser exposure: 1s
Power: 0.25mW
Spot size: 5µm^2
Rep. rate:250 kHz



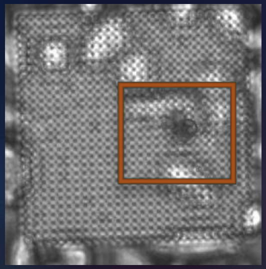
Before laser exposure

Microbubble formation in cells

Laser exposure: 1s
Power: 0.25mW
Spot size: 5µm^2
Rep. rate:250 kHz



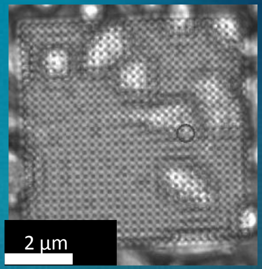
Before laser exposure



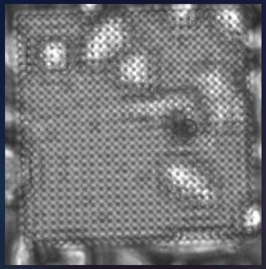
After laser exposure

Microbubble formation in cells

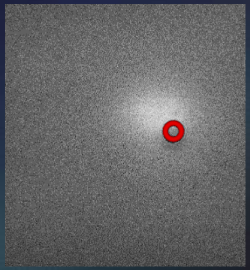
Laser exposure: 1s
Power: 0.25mW
Spot size: 5µm²
Rep. rate:250 kHz



Before laser exposure



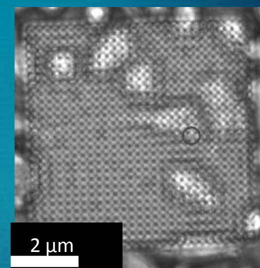
After laser exposure



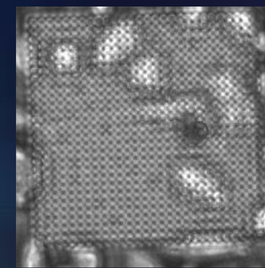
APG-2 Fluorescence

Microbubble formation in cells

Laser exposure: 1s
Power: 0.25mW
Spot size: 5µm^2
Rep. rate:250 kHz

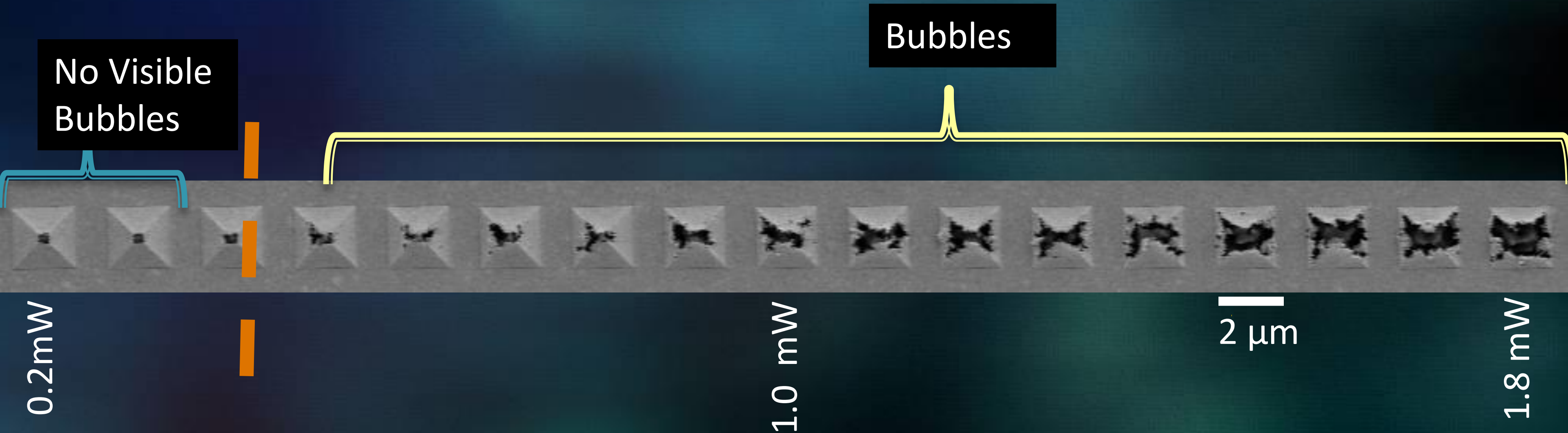


Before laser exposure

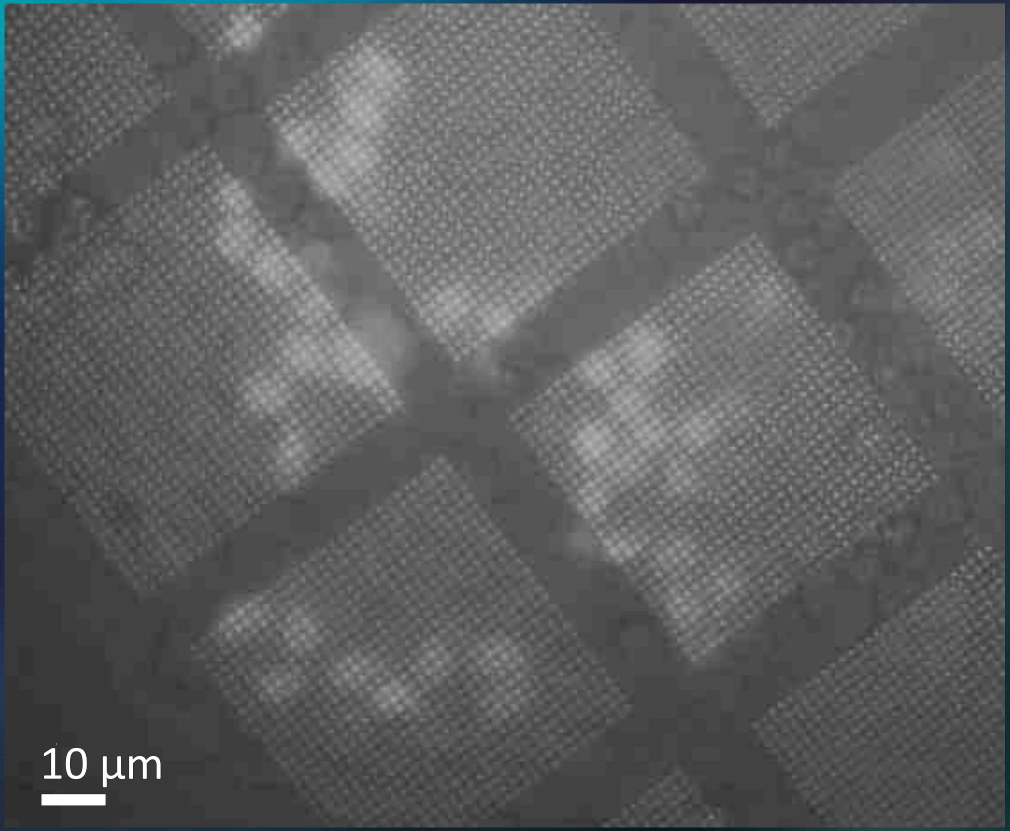


After laser exposure

APG-2 Fluorescence



Need to explore membrane-plasmon dynamics



Conclusion

Designed and fabricated a plasmonic substrate with high near-field enhancement

Cavitation (micro)bubbles damage substrate

Plasma membrane changes the cavitation bubble dynamics

Future Outlook

Understand membrane-plasmon dynamics

Explore laser parameters

Increase efficiency of poration

Perform transfection

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Thank you for your attention