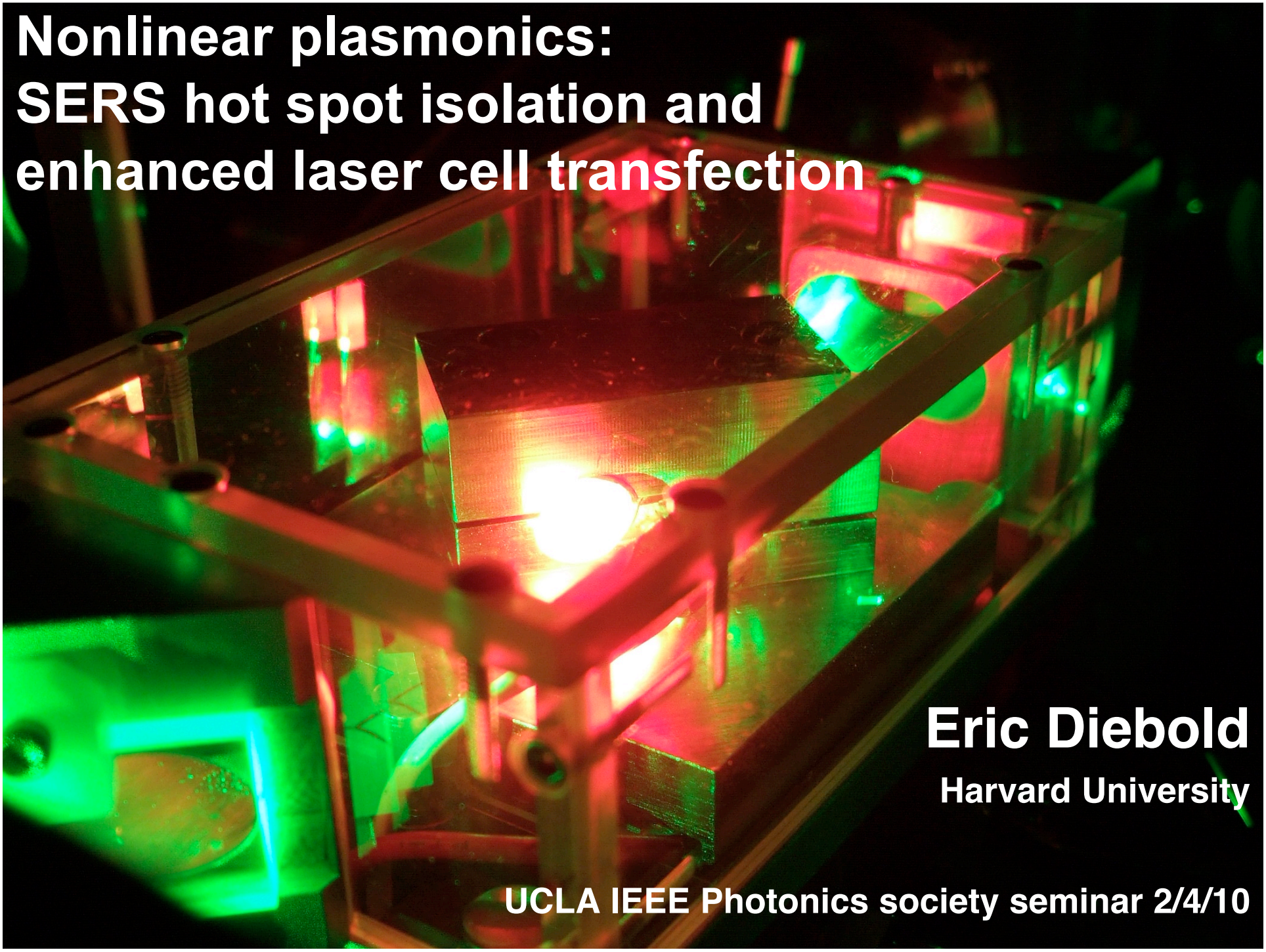
Nonlinear plasmonics: SERS hot spot isolation and enhanced laser cell transfection



Eric Diebold

Harvard University

UCLA IEEE Photonics society seminar 2/4/10

Outline

SERS hot spot isolation:

Background

Motivation: hot spot distribution

Hot spot isolation

Plasmon-enhanced laser cell transfection:

Background: femtosecond laser cell transfection

Motivation: plasmonic substrates

Ultrafast plasmon-cell interactions

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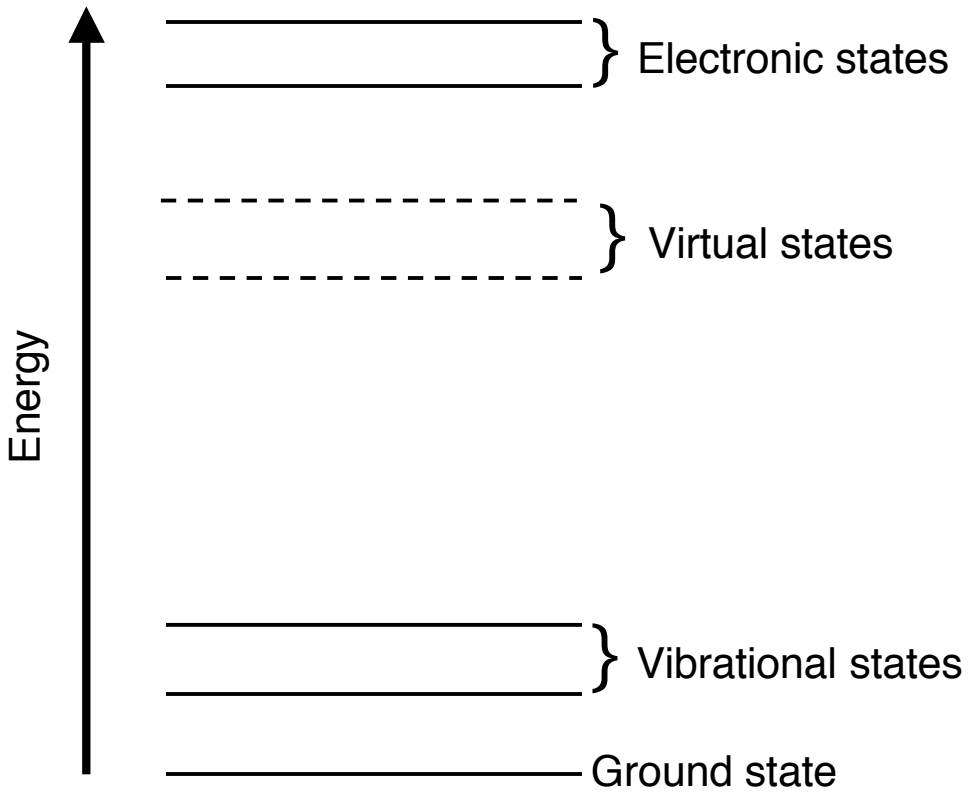

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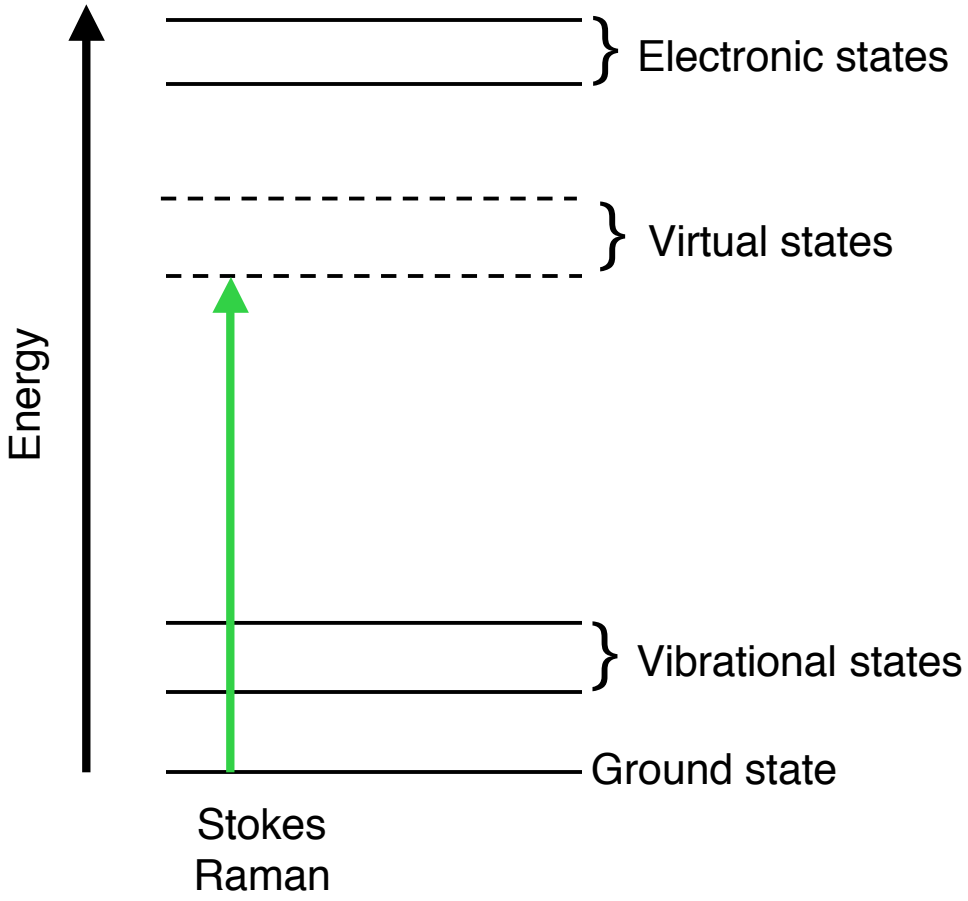

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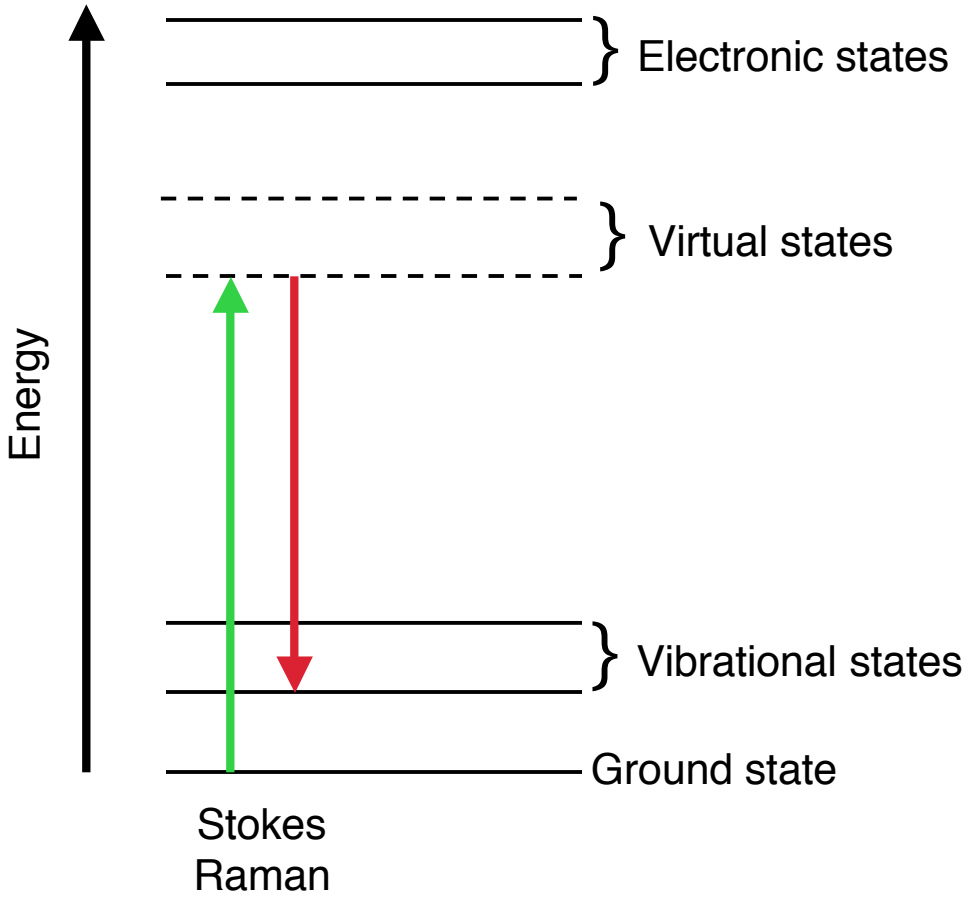

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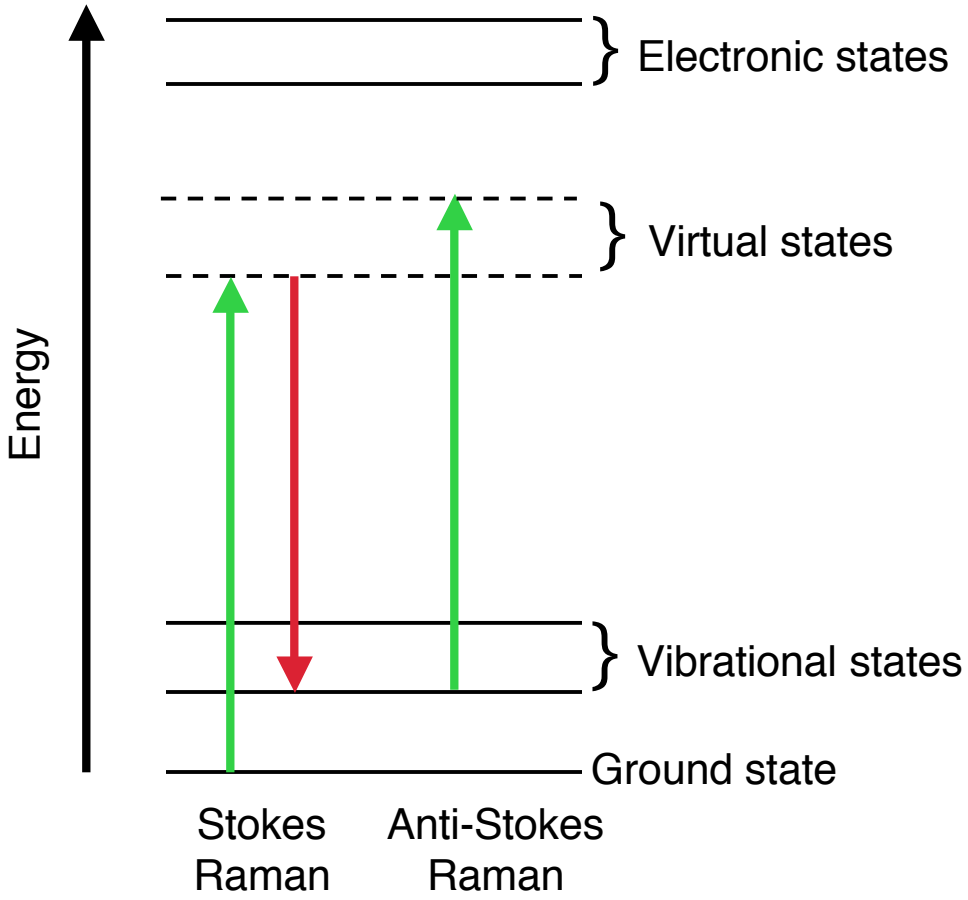

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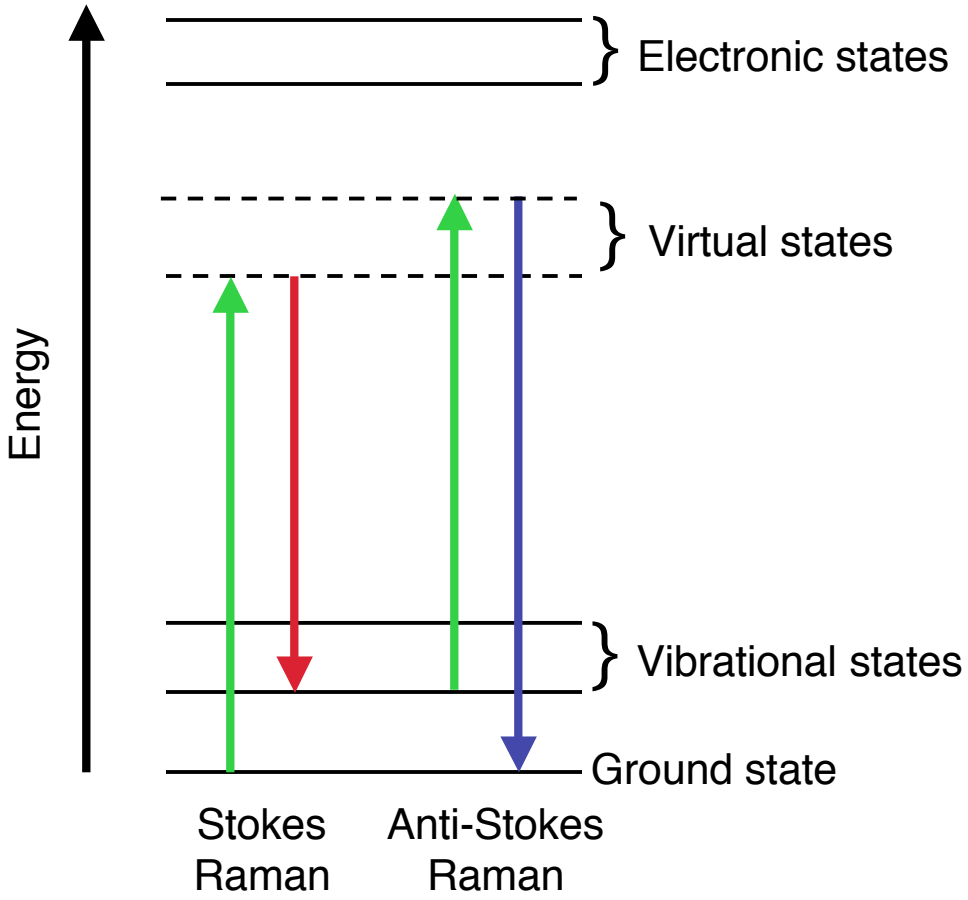

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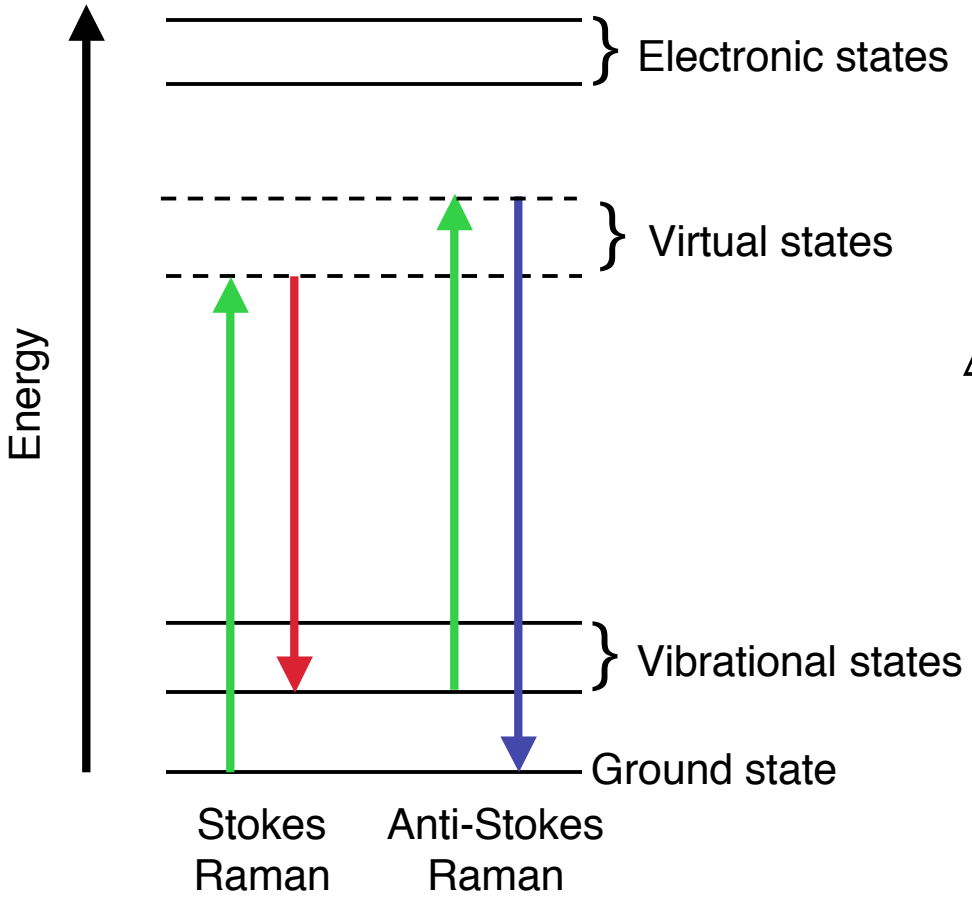
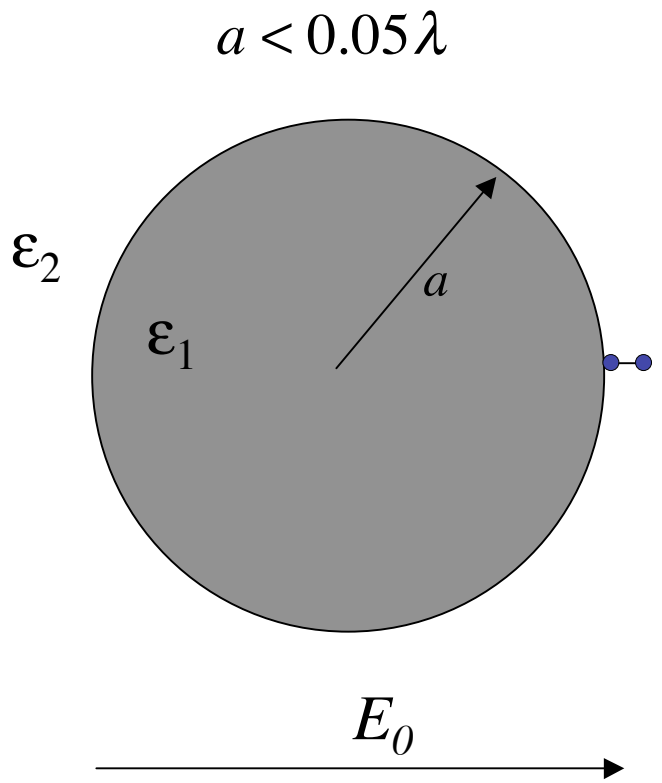


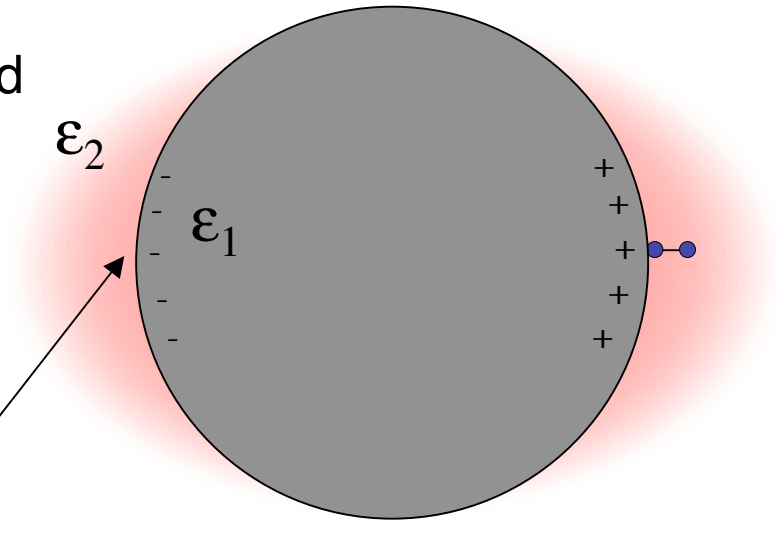
$$\Delta\upsilon = \frac{1}{\lambda_{incident}} - \frac{1}{\lambda_{scattered}}$$

Background



Background

1. Near-field scattered electric field enhances polarization of molecules located near surface



$$\frac{|E_s|}{|E_0|} \propto \frac{\varepsilon_1(\omega) - \varepsilon_2}{\varepsilon_1(\omega) + 2\varepsilon_2}$$

E_0

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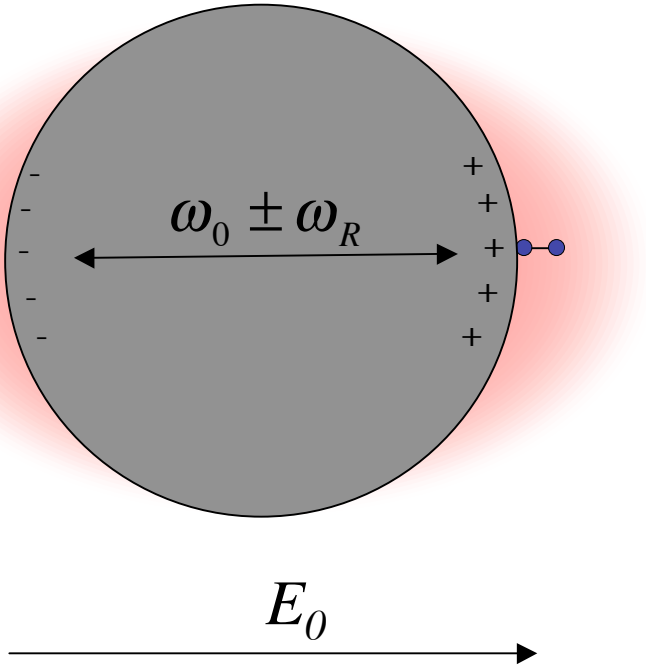
1. Near-field scattered electric field enhances polarization of molecules located near surface

2. Field from molecular polarization generates polarization of sphere at Raman frequency



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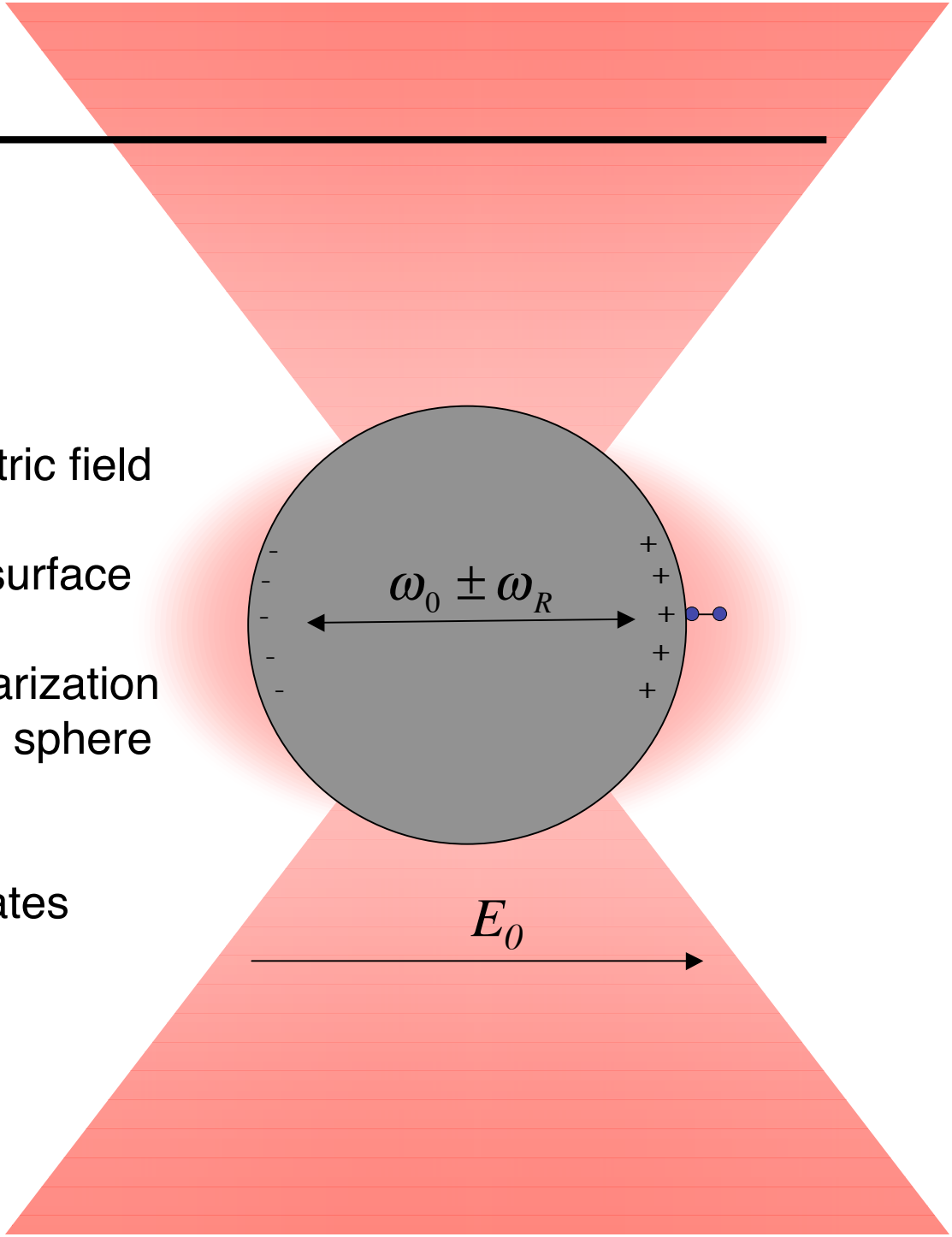
3. Sphere polarization radiates Raman field into far field



Background

Electromagnetic SERS enhancement factor:

$$\frac{I_{\text{SERS}}}{I_{\text{Normal Raman}}} \propto \left(\frac{\left|E_s\left(\omega_0\right)\right|}{\left|E_0\left(\omega_0\right)\right|} \right)^2 \times \left(\frac{\left|E_s\left(\omega_0 - \omega_R\right)\right|}{\left|E_0\left(\omega_0 - \omega_R\right)\right|} \right)^2 \approx \left(\frac{\left|E_s\left(\omega_0\right)\right|}{\left|E_0\left(\omega_0\right)\right|} \right)^4$$

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$$\sigma' \simeq \sigma \left| \frac{\varepsilon_1(\omega) - \varepsilon_2}{\varepsilon_1(\omega) + 2\varepsilon_2} \right|^2 \left| \frac{\varepsilon_1(\omega) - \varepsilon_2}{\varepsilon_1(\omega) + 2\varepsilon_2} \right|^2 \left(\frac{a}{a + r} \right)^{12}$$

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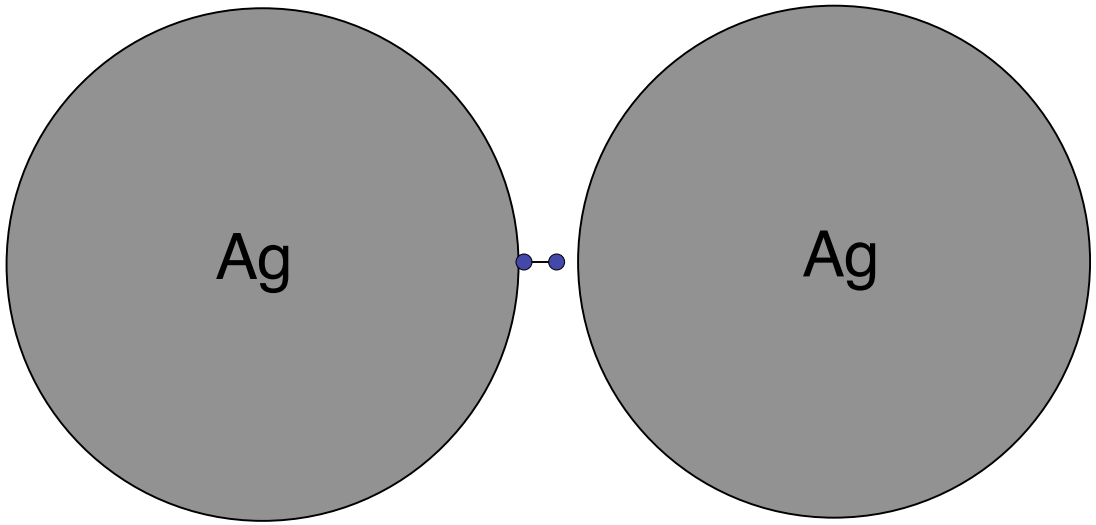

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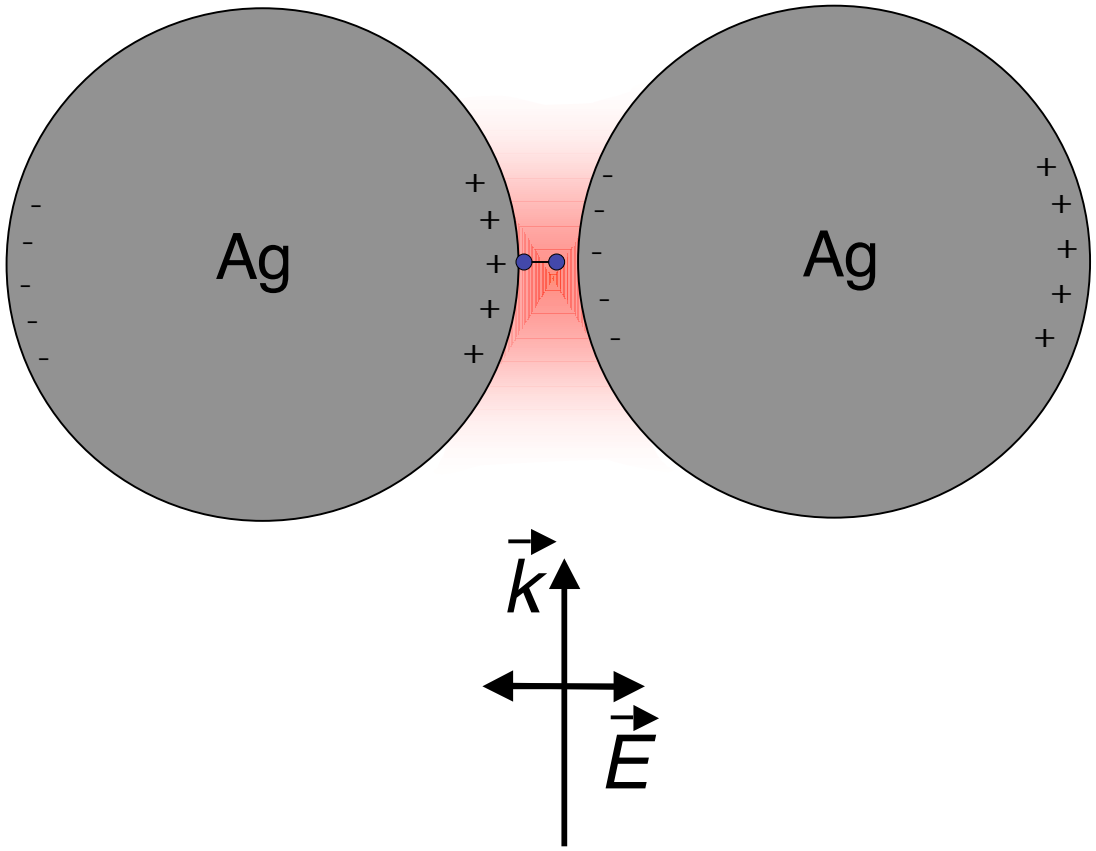

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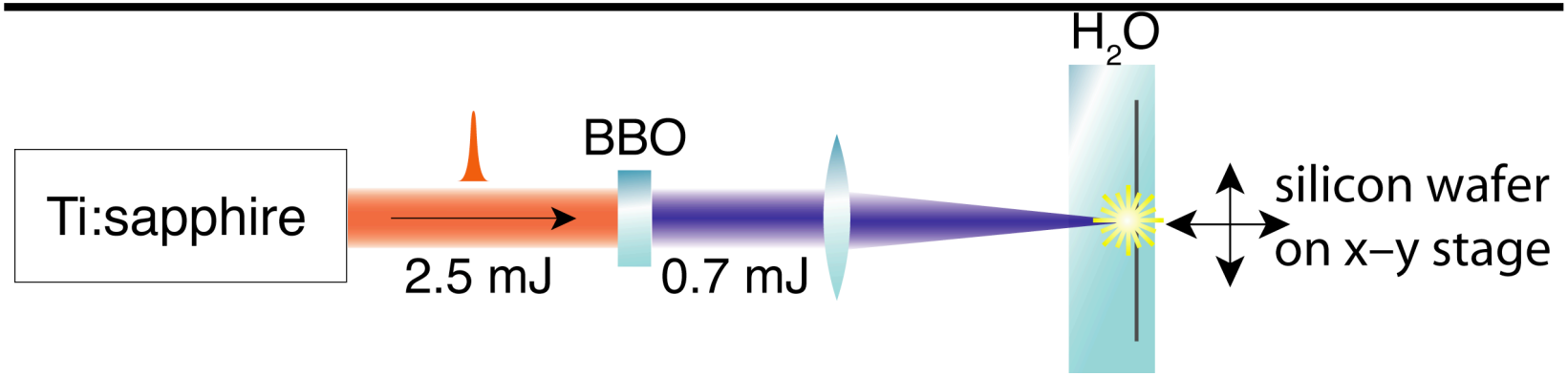
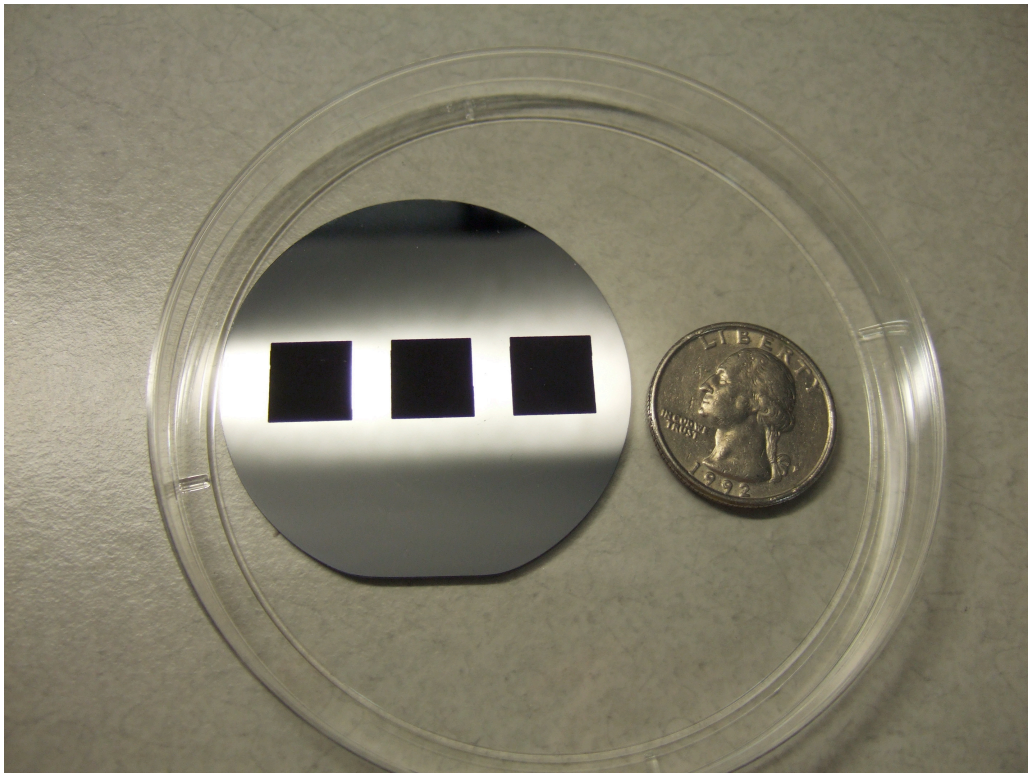
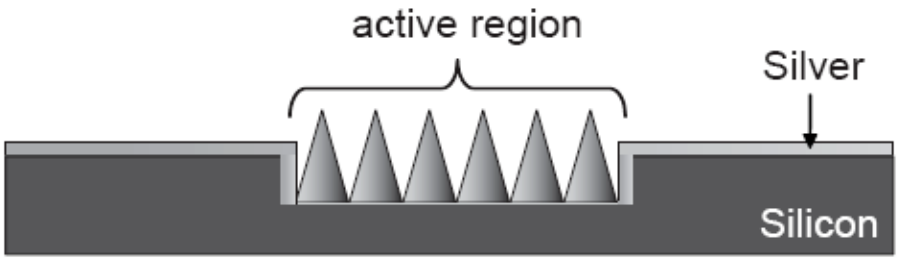




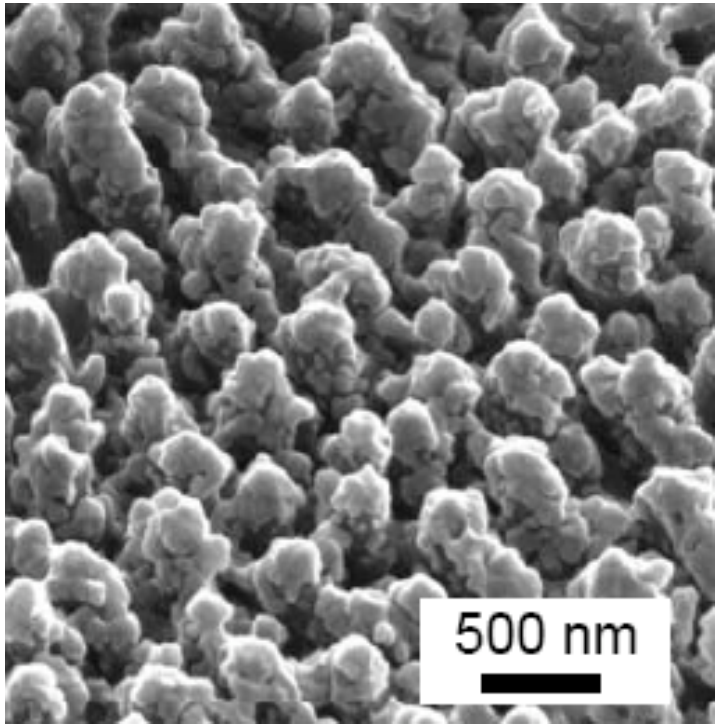
E.D. Diebold, et al. *Langmuir* **25**, 1790 (2009)

Background

1. Femtosecond laser structuring
2. Thermal evaporation - 80nm Ag



Active region



Average enhancement factor (benzenethiol) ~ 10^7

E.D. Diebold, et al. *Langmuir* **25**, 1790 (2009)

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Measurement of the Distribution of Site Enhancements in Surface-Enhanced Raman Scattering

Ying Fang, Nak-Hyun Seong, Dana D. Dlott

Raman enhancement factor η	Percentage of molecules	Percentage contribution to overall SERS signal
$<2.8 \times 10^4$	0	0
2.8×10^4 to 1×10^5	61%	4%
10^5 to 10^6	33%	11%
10^6 to 10^7	5.1%	16%
10^7 to 10^8	0.7%	22%
10^8 to 10^9	0.08%	23%
10^9 to 10^{10}	0.006%	17%
$>10^{10}$	0.0003%	7%

Fang, et al. *Science* **381**, 288 (2008)

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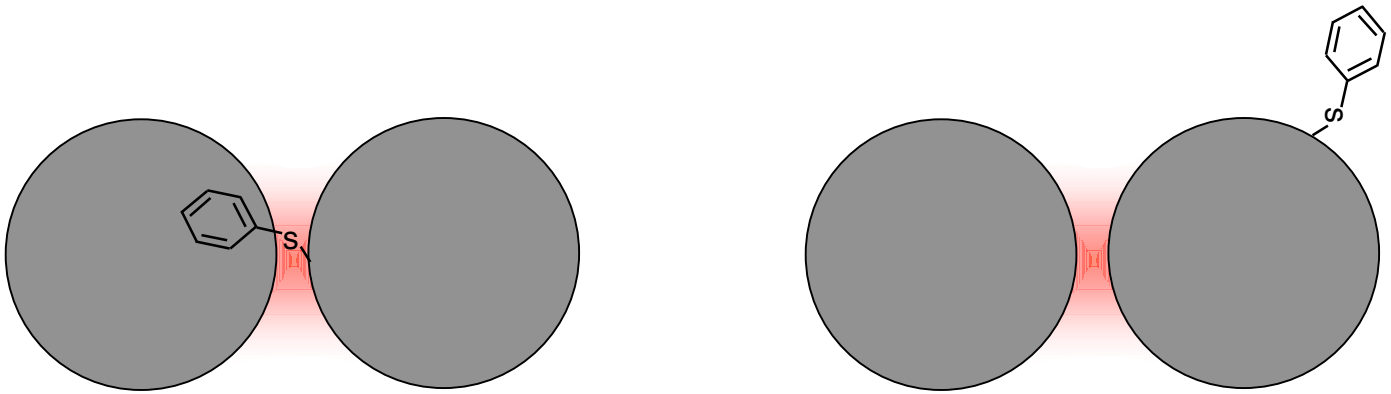
Only **63** out of **1,000,000** sites are "hot spots" (EF > 10^9), yet their contribution to the total SERS signal is 24%!

Fang, et al. *Science* **381**, 288 (2008)

Motivation: hot spot distribution

If $N_{analyte}$ is small, how do we ensure that molecules adsorb only to hot spots?



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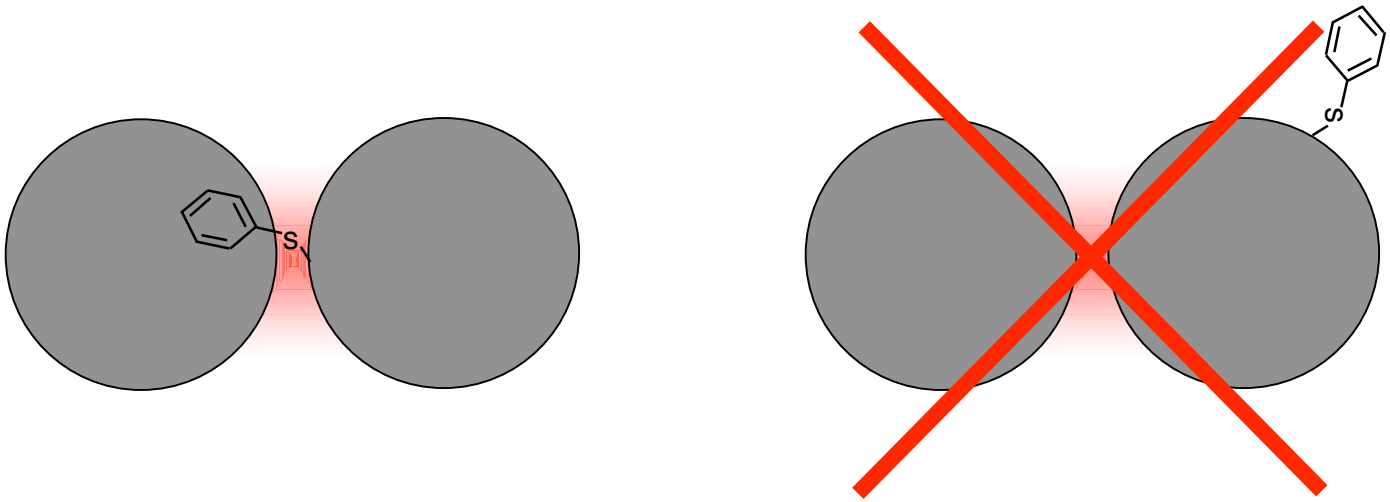

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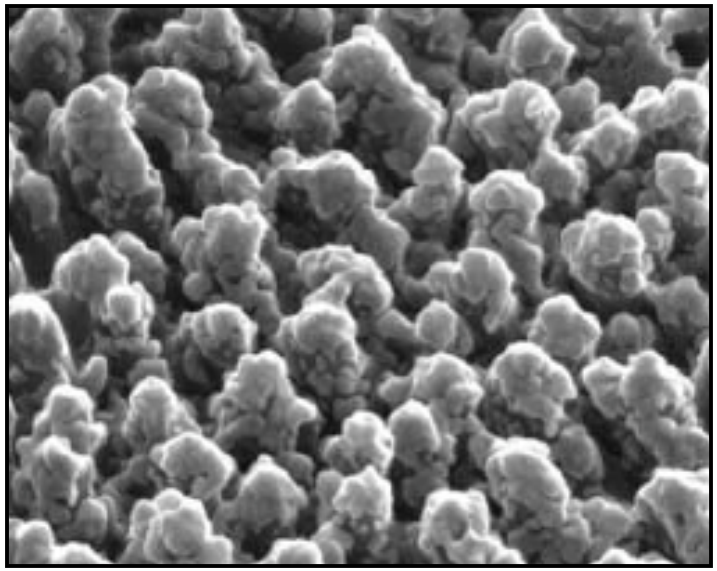

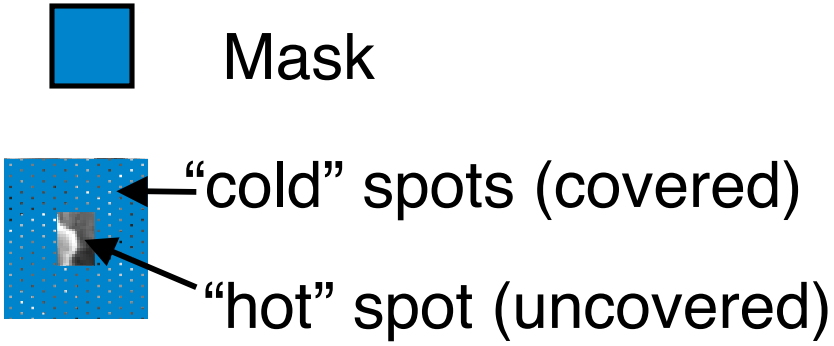

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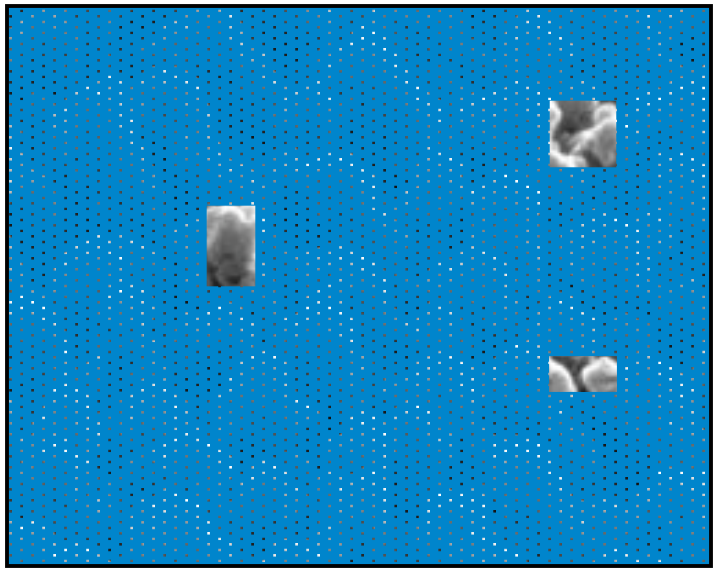

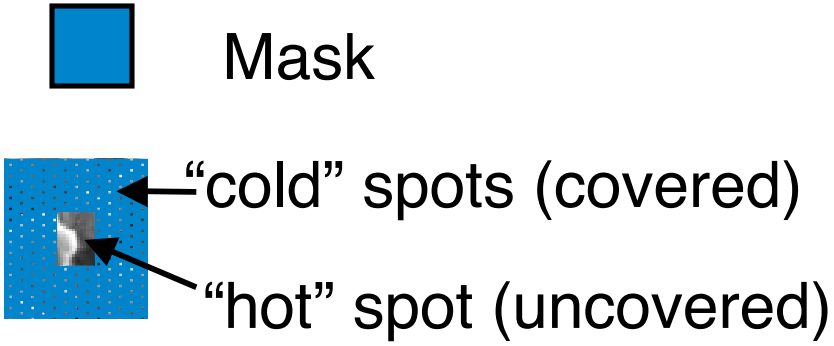

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Background: laser nanostructured substrates

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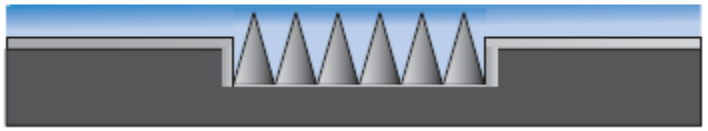
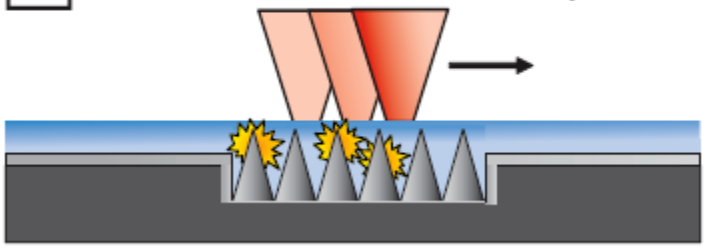
Hot spot isolation

1 Spin coat positive-tone resist



Shipley S1805 photoresist
(~30nm thick layer)

2 Femtosecond-laser exposure



Multiphoton-induced luminescence from Ag hot spots exposes photoresist

3 Development

Developer removes exposed areas, uncovering hot spots

Diebold et al., *J. Am. Chem. Soc.*, **131**, 16356-16357 (2009)

Hot spot isolation

HSI substrates expected to show higher enhancement
under conditions of sub-monolayer coverage.

$$N_{analyte} << N_{adsorption\ sites}$$

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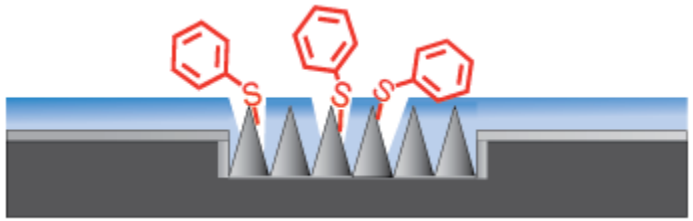
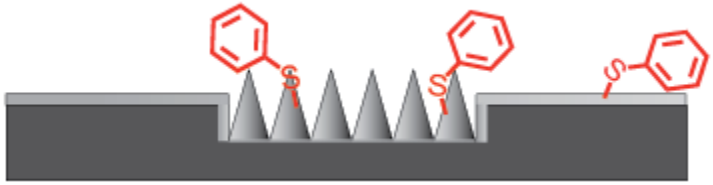


HSI-SERS substrate

Analyte binds exclusively
to exposed hot spots



SERS substrate

Analyte distributed over
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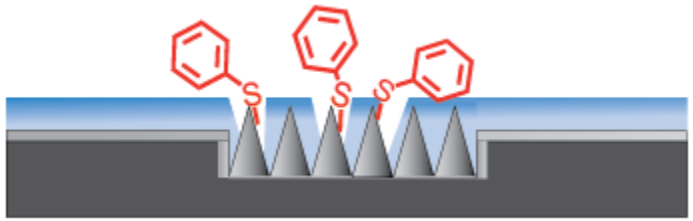

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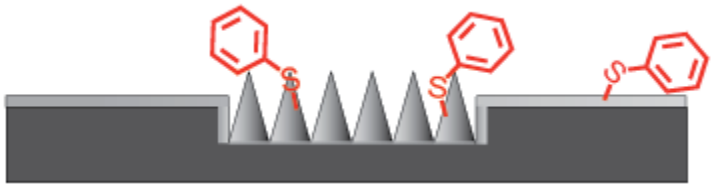

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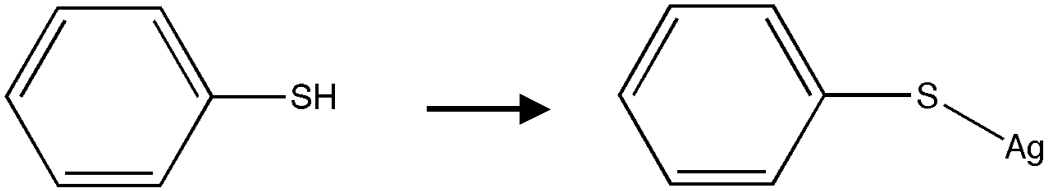
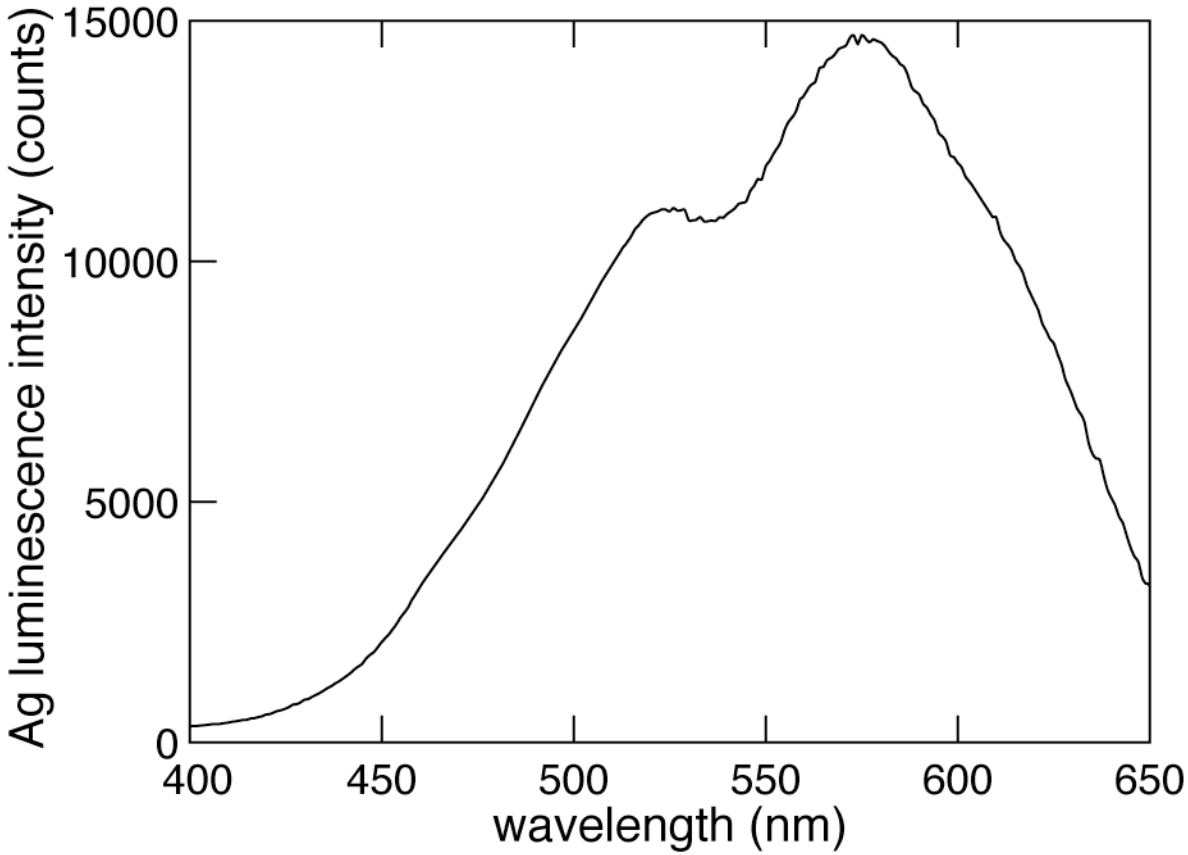


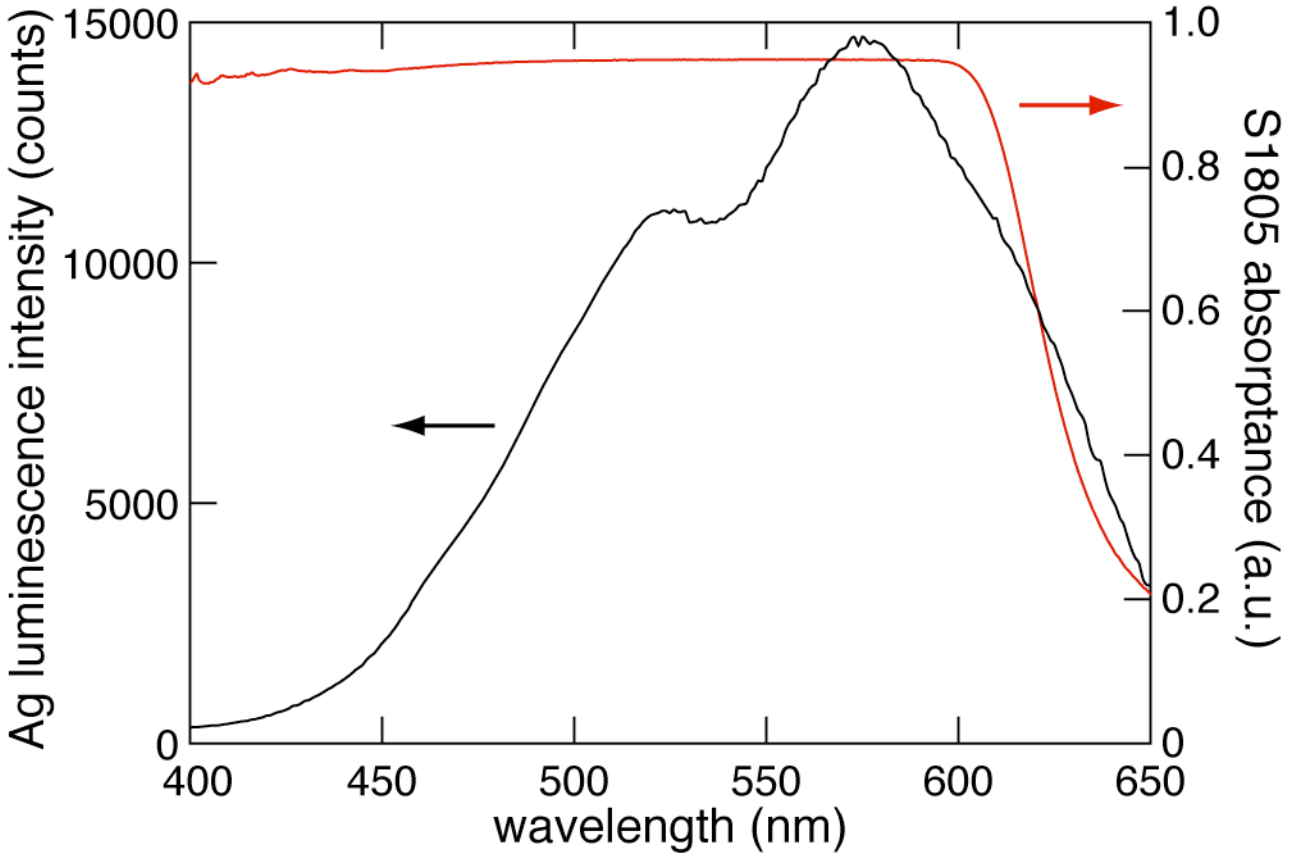
Benzenethiol

Hot spot isolation



λ_{center} = 795nm, τ = 60fs, 100 pulses/spot

Hot spot isolation



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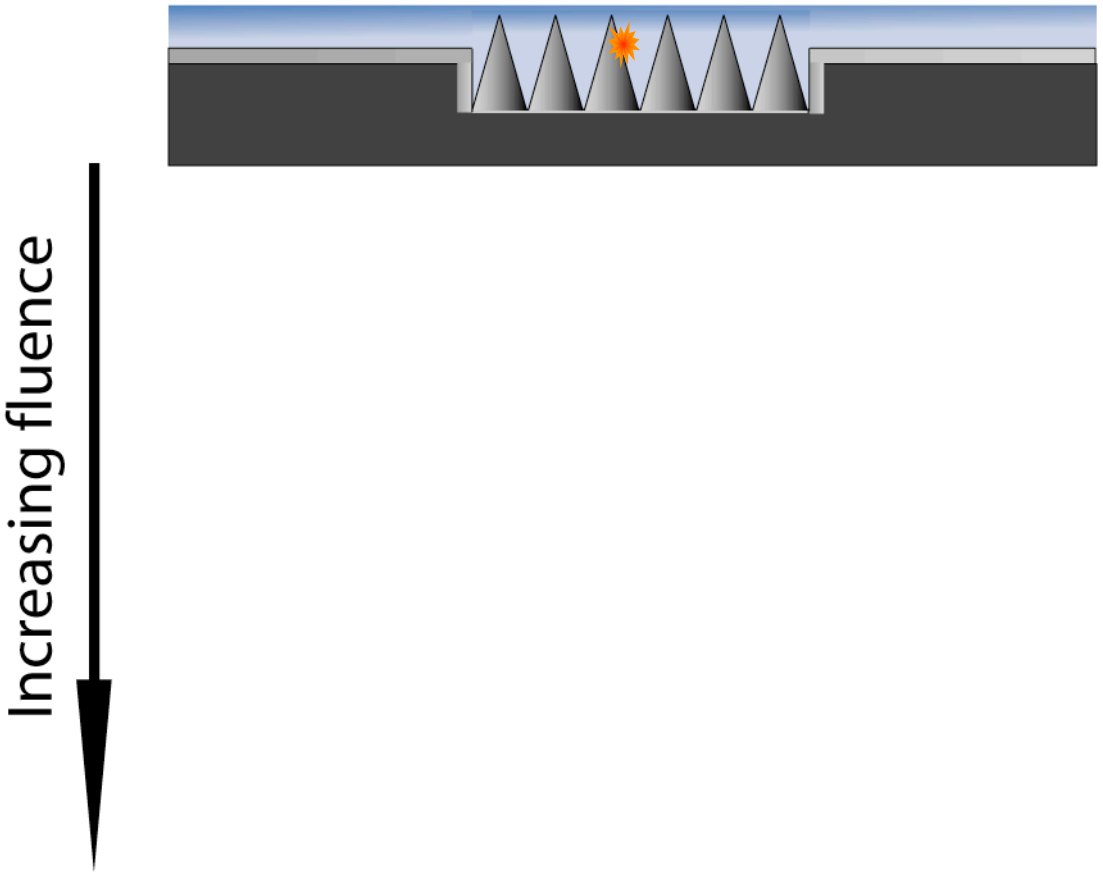

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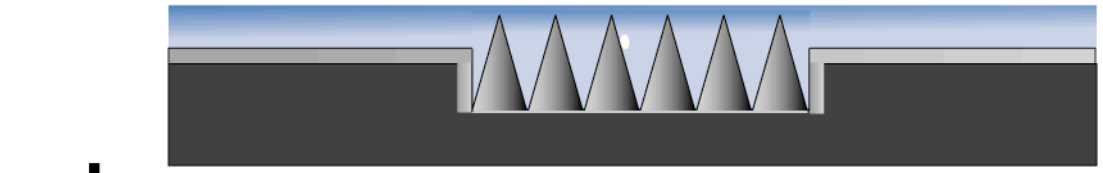

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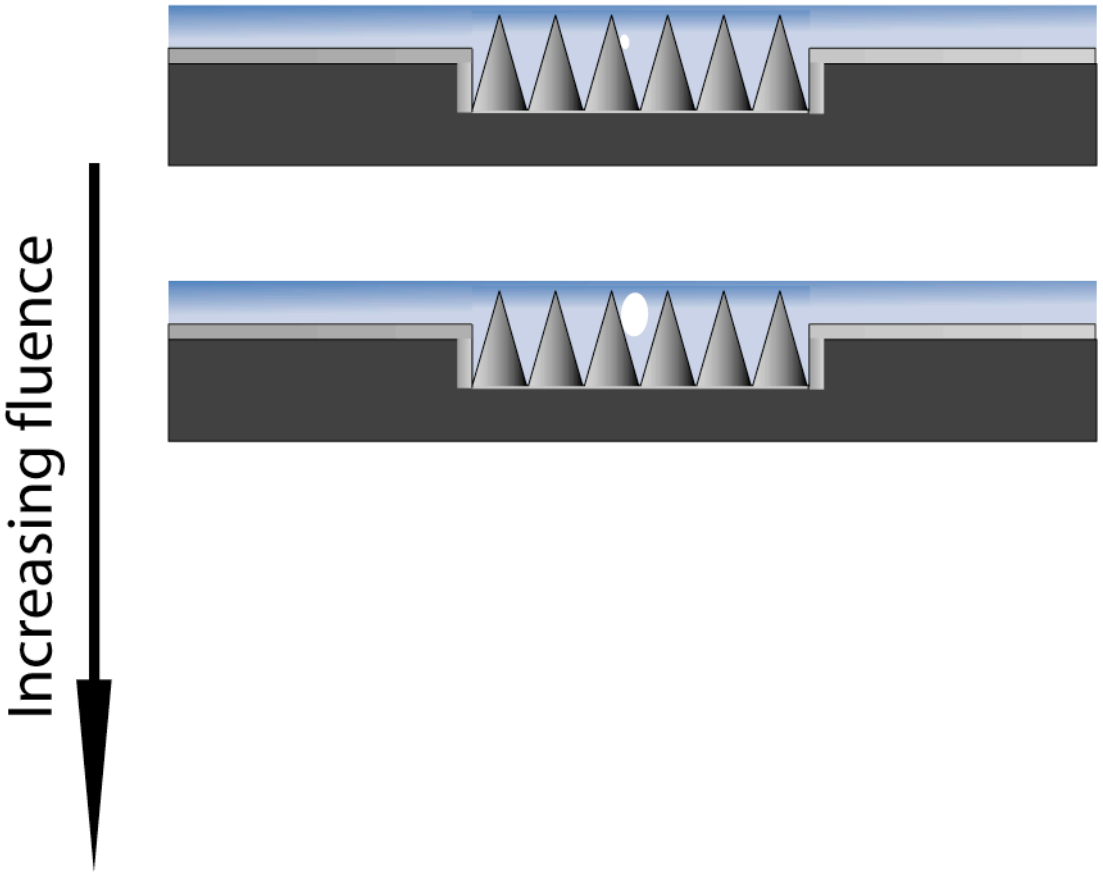

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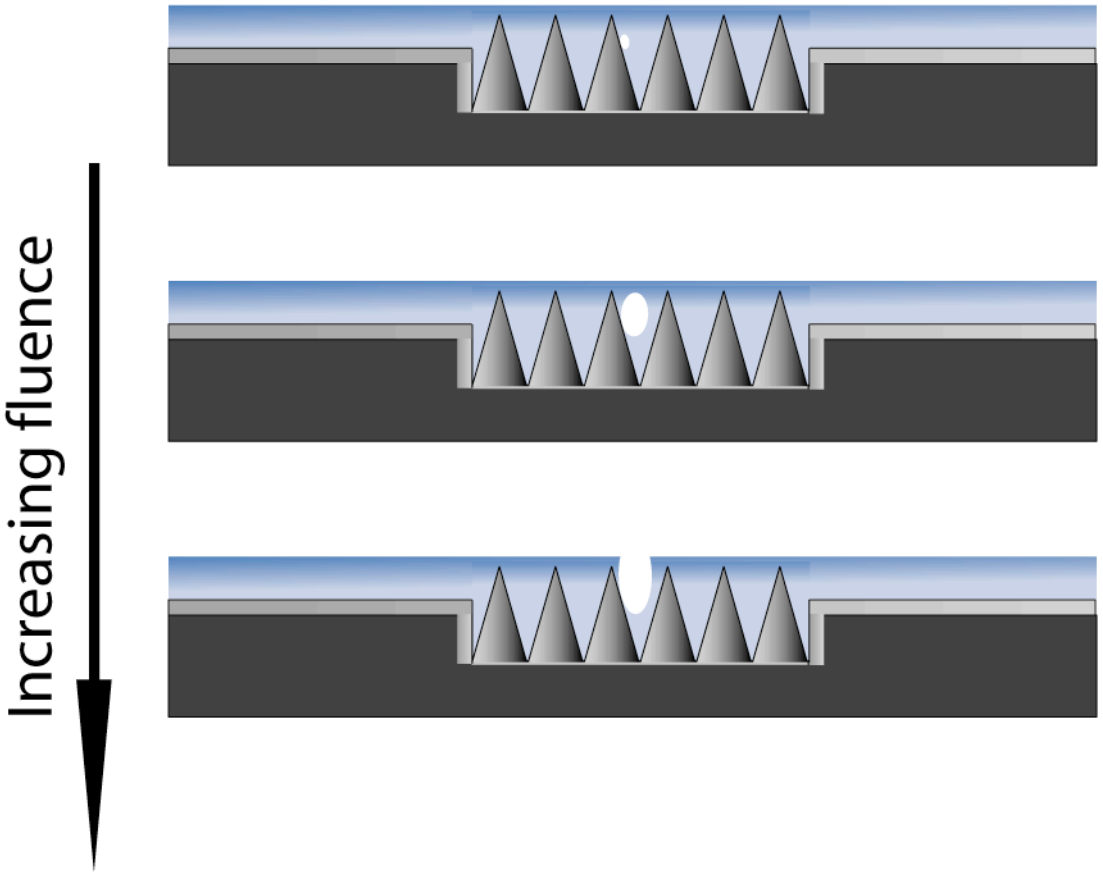

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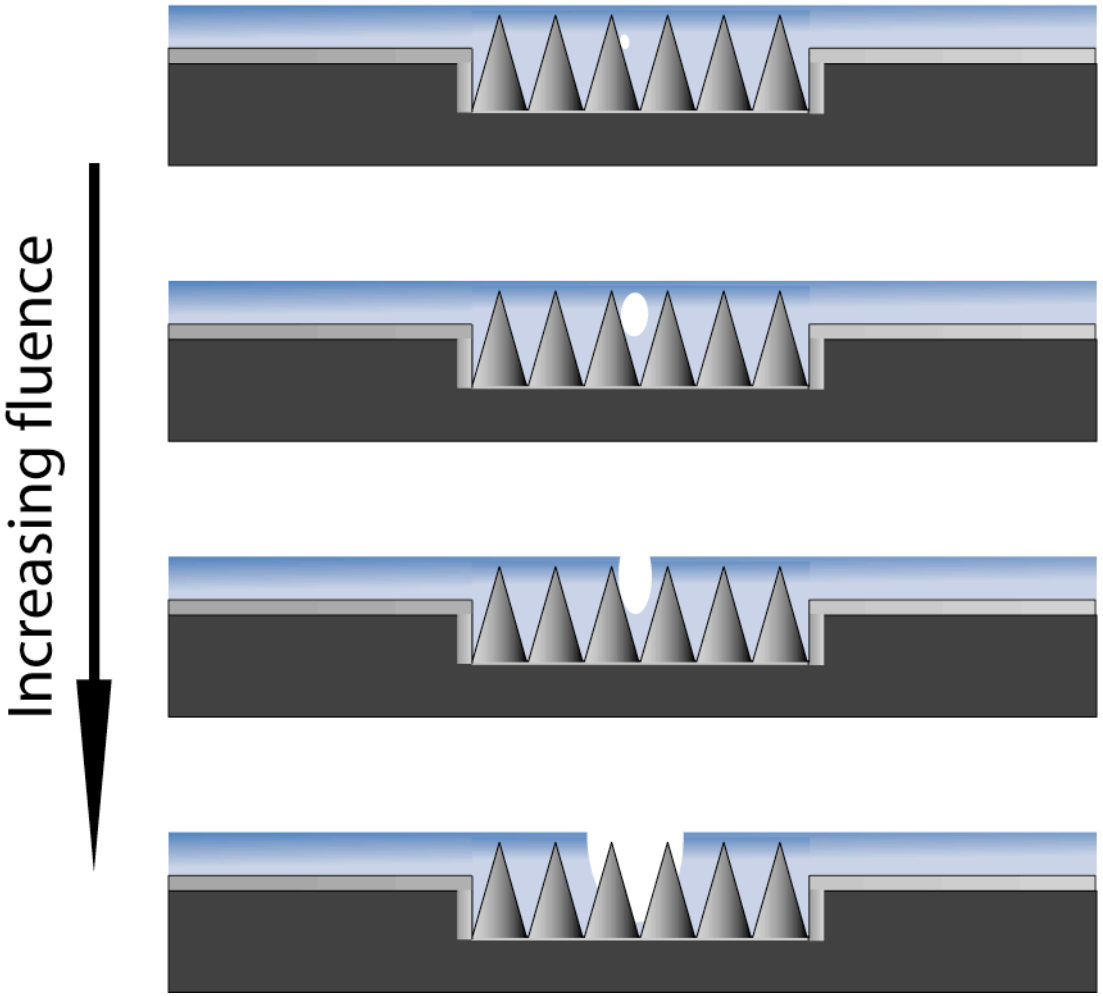

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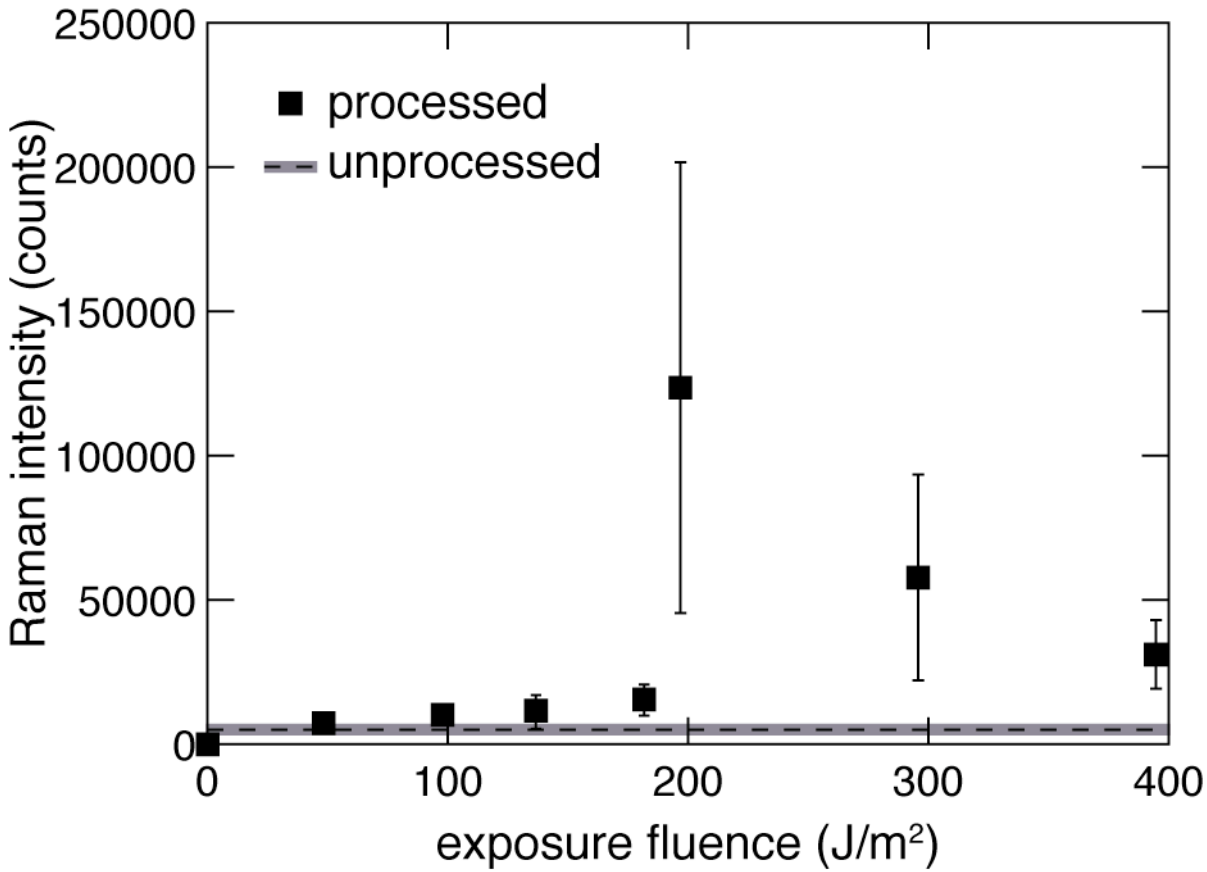
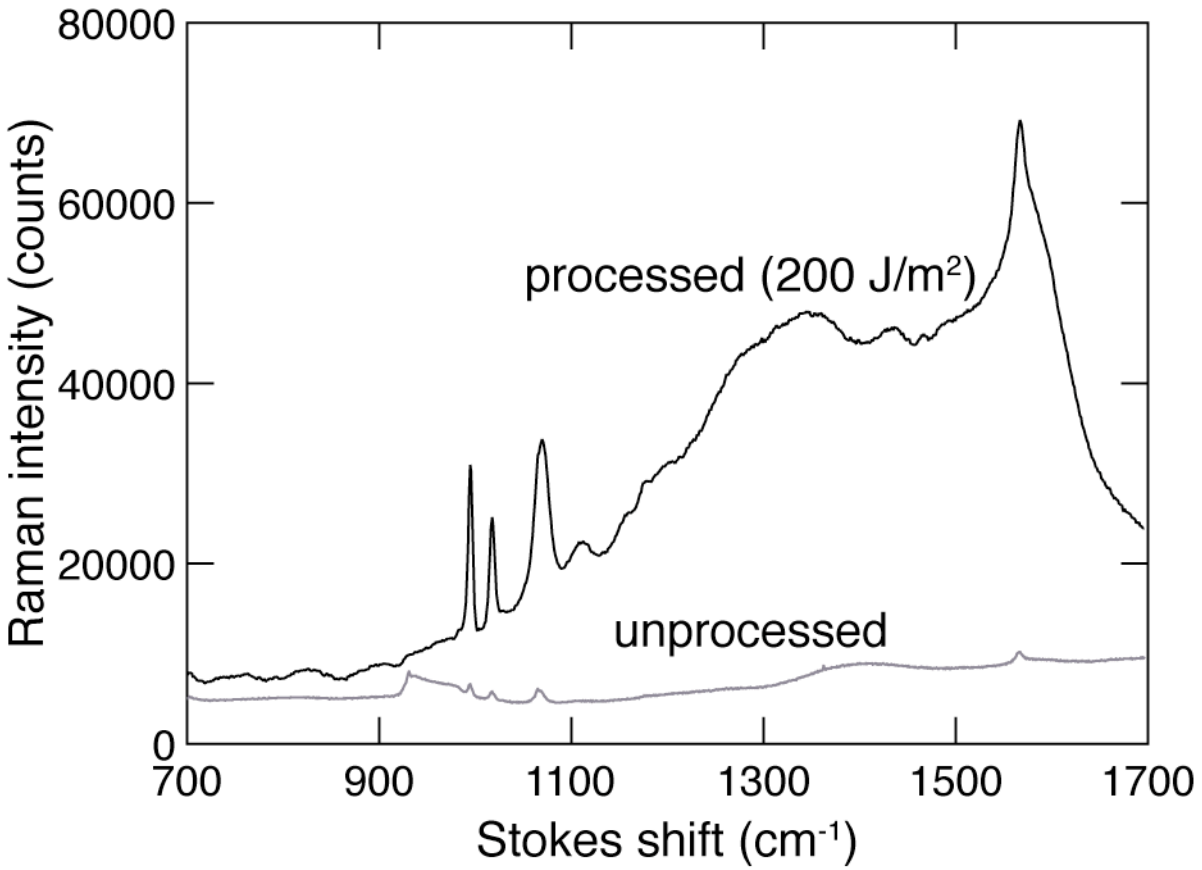


24-hour incubation with 4 femtomoles of benzenethiol
12mW, 785nm excitation, 30s integration, 0.40NA objective

Diebold et al., *J. Am. Chem. Soc.*, **131**, 16356-16357 (2009)

Hot spot isolation



27× times signal improvement (998 cm^{-1} band)

Diebold et al., *J. Am. Chem. Soc.*, **131**, 16356-16357 (2009)

Hot spot isolation

Average enhancement factor:

Submonolayer coverage:

24 hour incubation with 2.4 x 10^9 molecules = 0.001% surface coverage.

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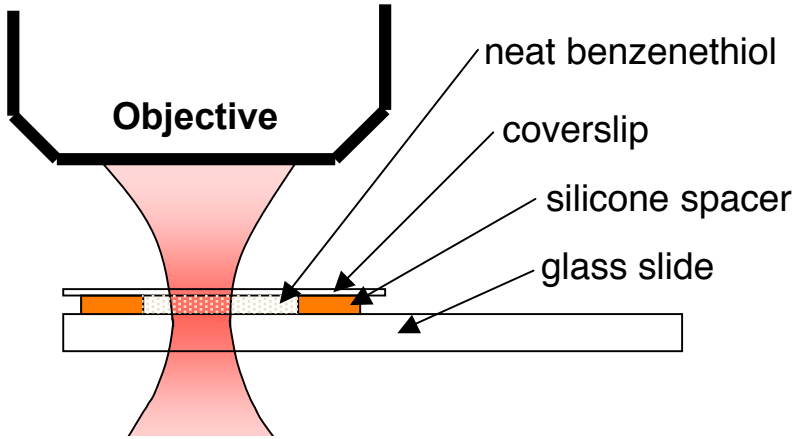
Signal normalized to neat benzenethiol using confocal microscope method.

$$EF = \frac{I_{SERS}}{I_{Neat}} \frac{N_{Neat}}{N_{SERS}}$$



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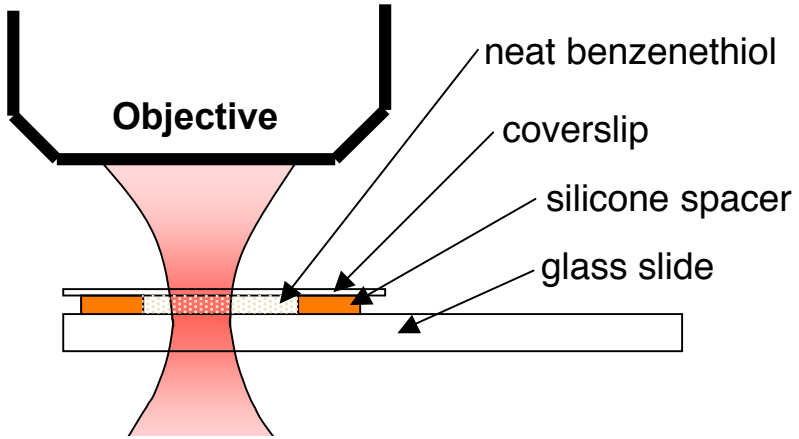

Enhancement factor (998 cm^{-1}) = 3 x 10^9

Take home message

Hot spot isolation:

1. is generally applicable to noble metal SERS substrates and masks “cold spots,” allowing molecules to bind only to “hot spots.”

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Take home message

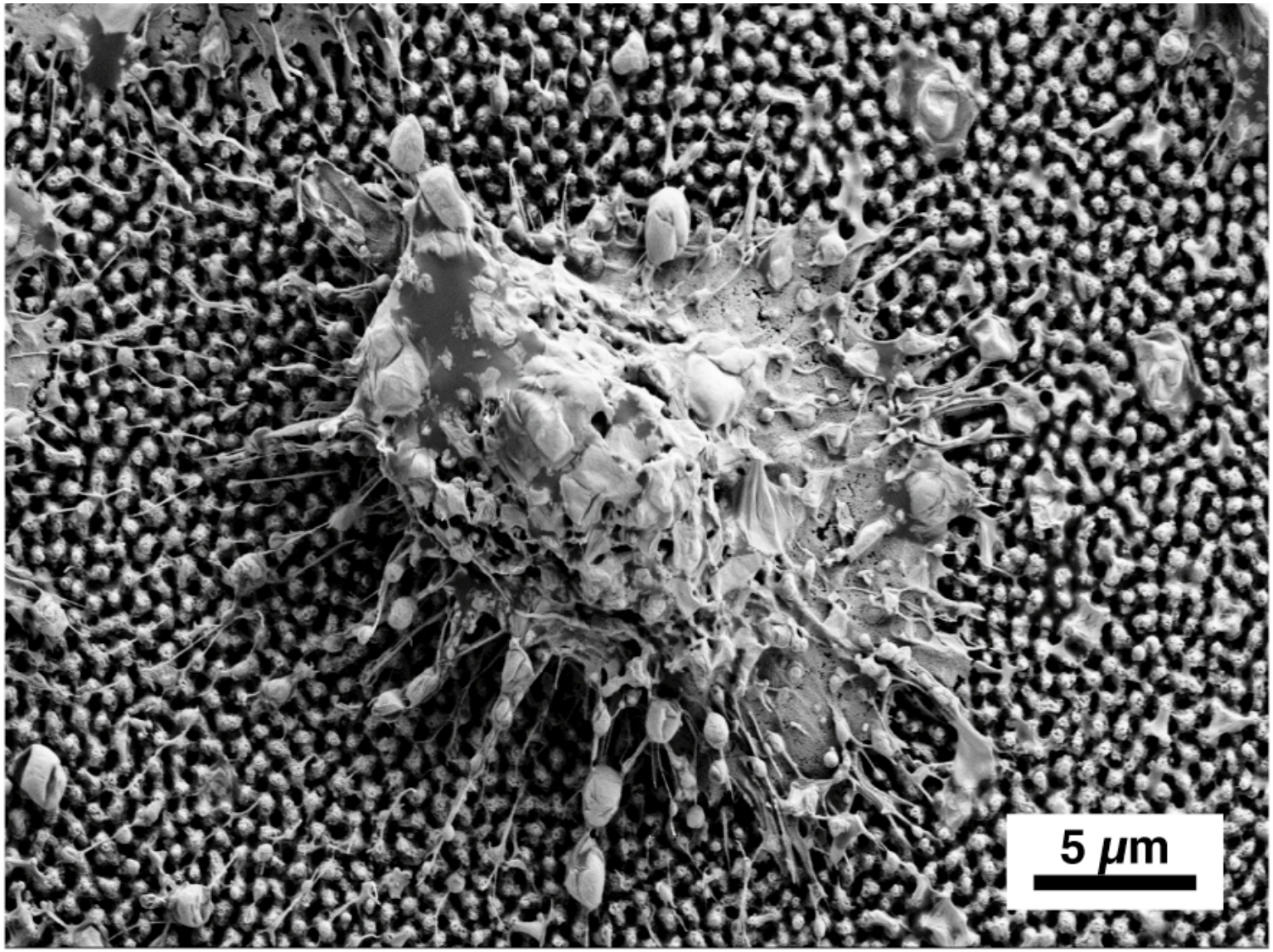
Hot spot isolation:

1. is generally applicable to noble metal SERS substrates and masks “cold spots,” allowing molecules to bind only to “hot spots.”

2. does not require knowledge of hot spot location or enhancement factor.

3. offers significant SERS signal improvement under sub-monolayer coverage.

Plasmon-enhanced laser cell transfection



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Motivation: hot spot distribution

Hot spot isolation

Plasmon-enhanced laser cell transfection:

Background: femtosecond laser cell transfection

Motivation: plasmonic substrates

Ultrafast plasmon-cell interactions

Background: femtosecond laser cell transfection

Cell transfection: “infection by transformation”

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

Background: femtosecond laser cell transfection

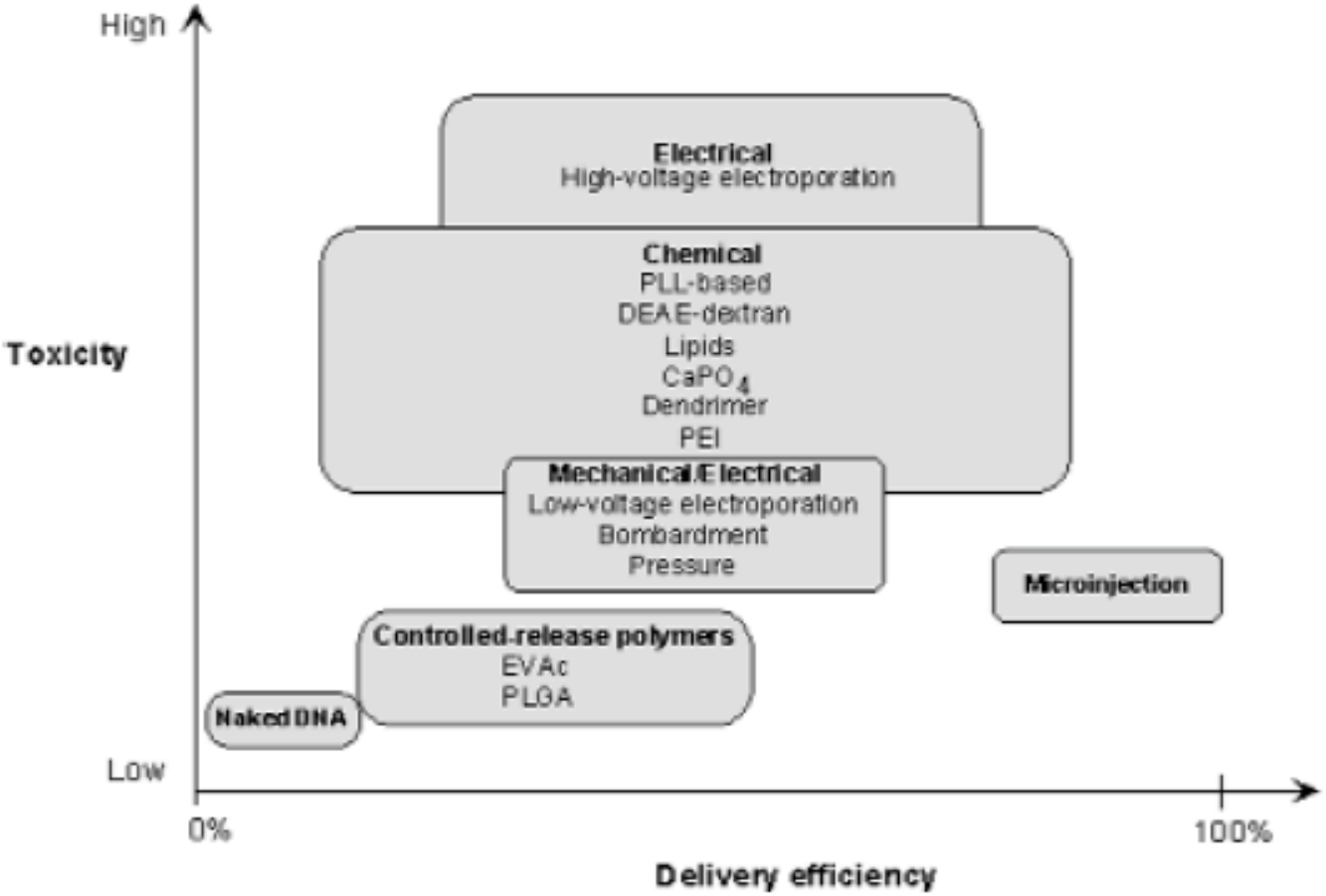
Cell transfection is central to:

Genetic engineering

Potential gene therapies - DNA, siRNA, etc.

Basic biological research

Background: femtosecond laser cell transfection



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

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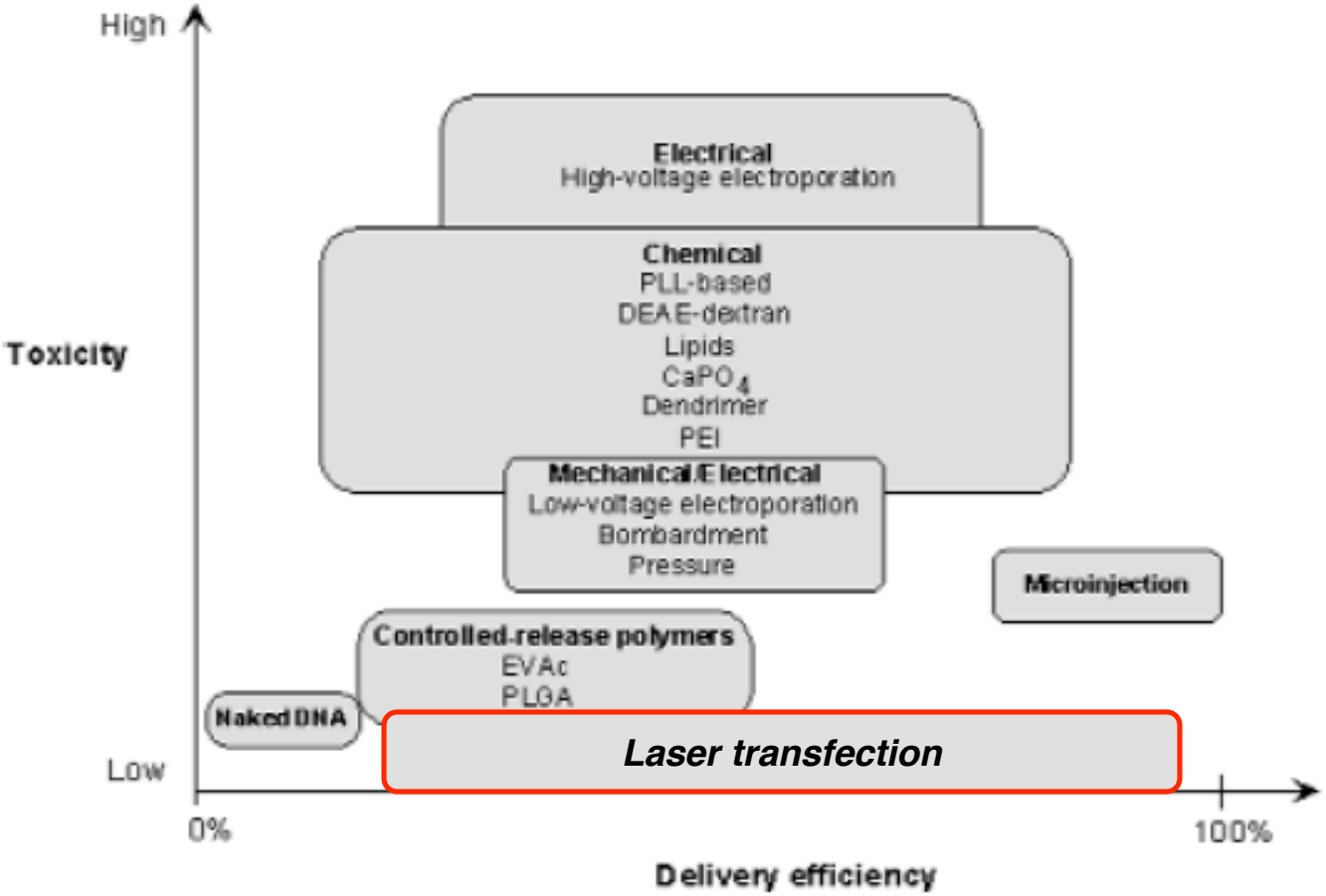

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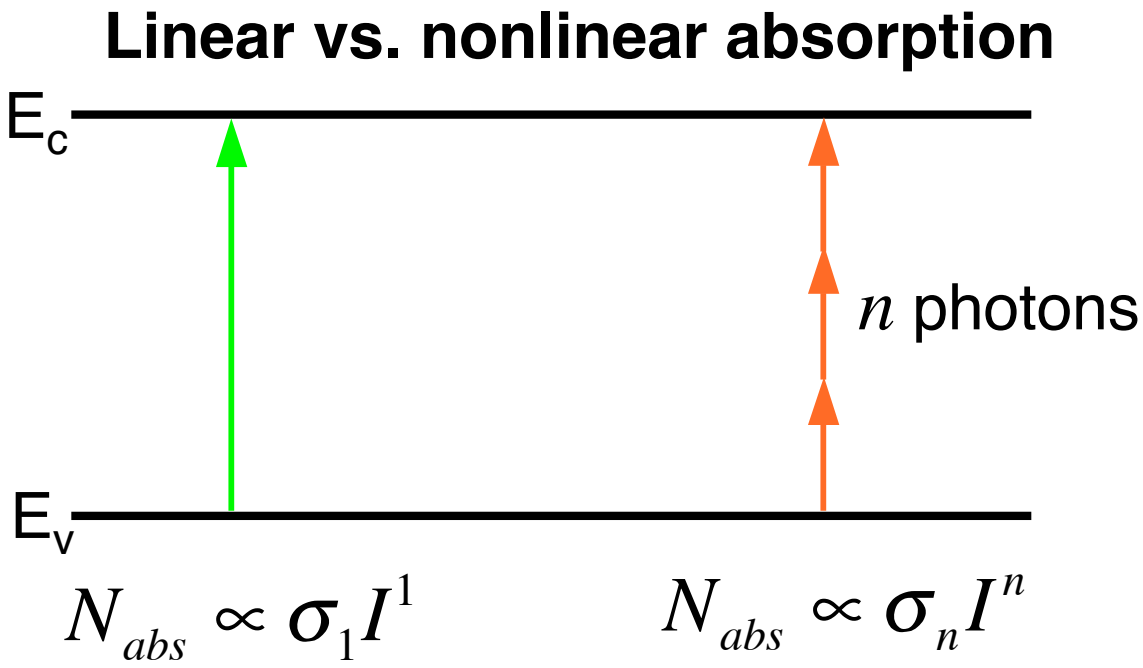

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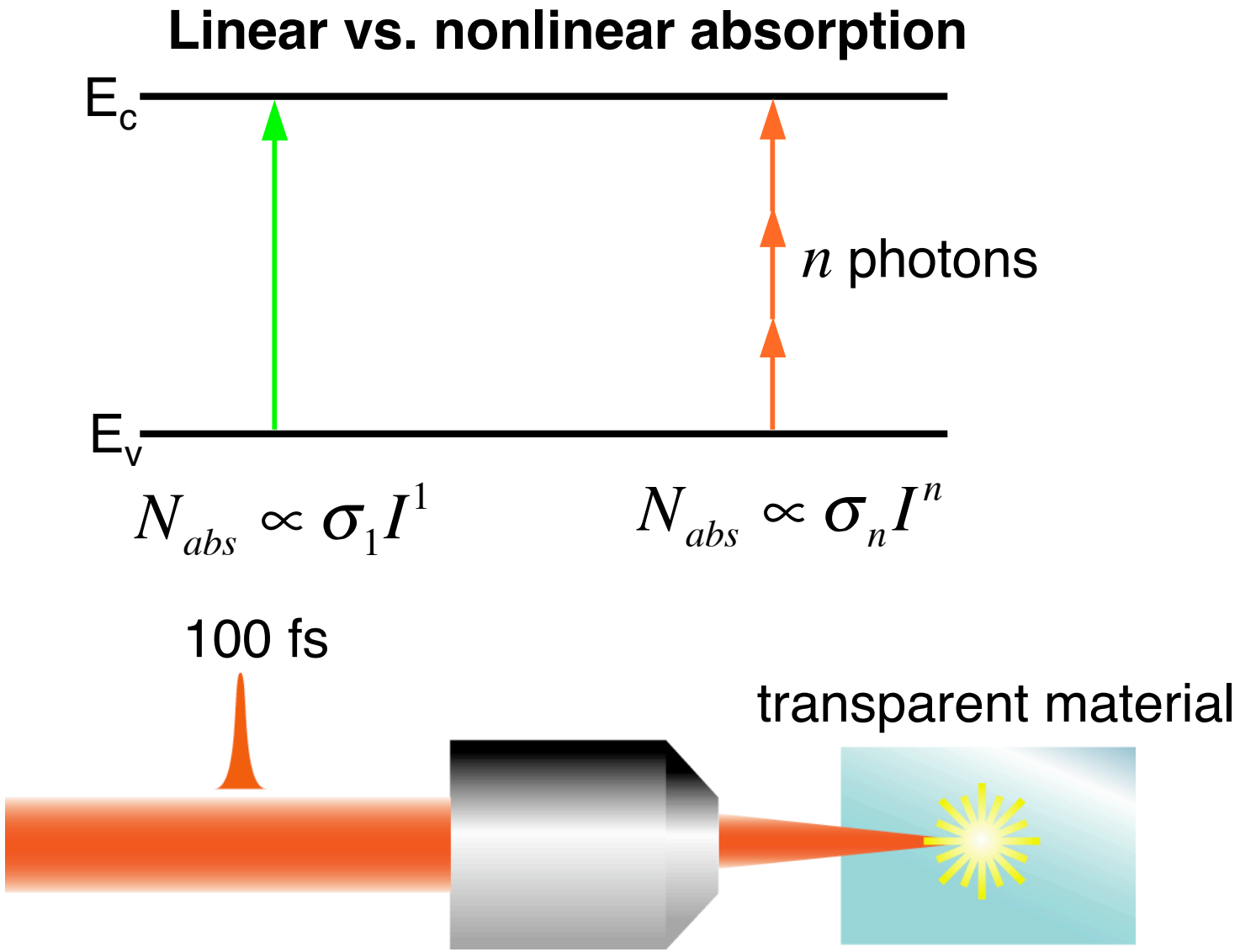

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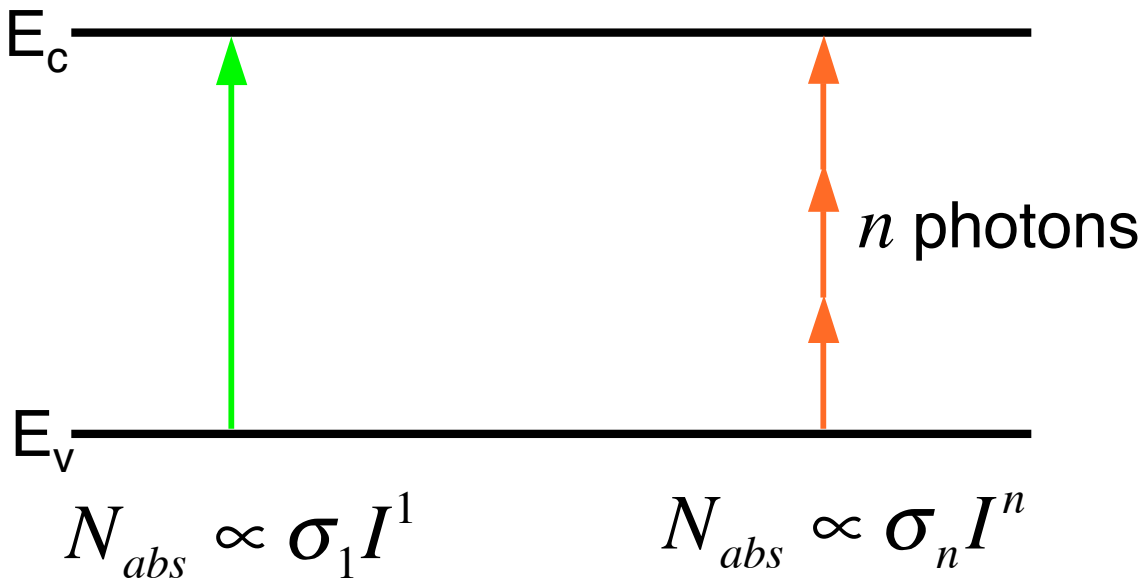
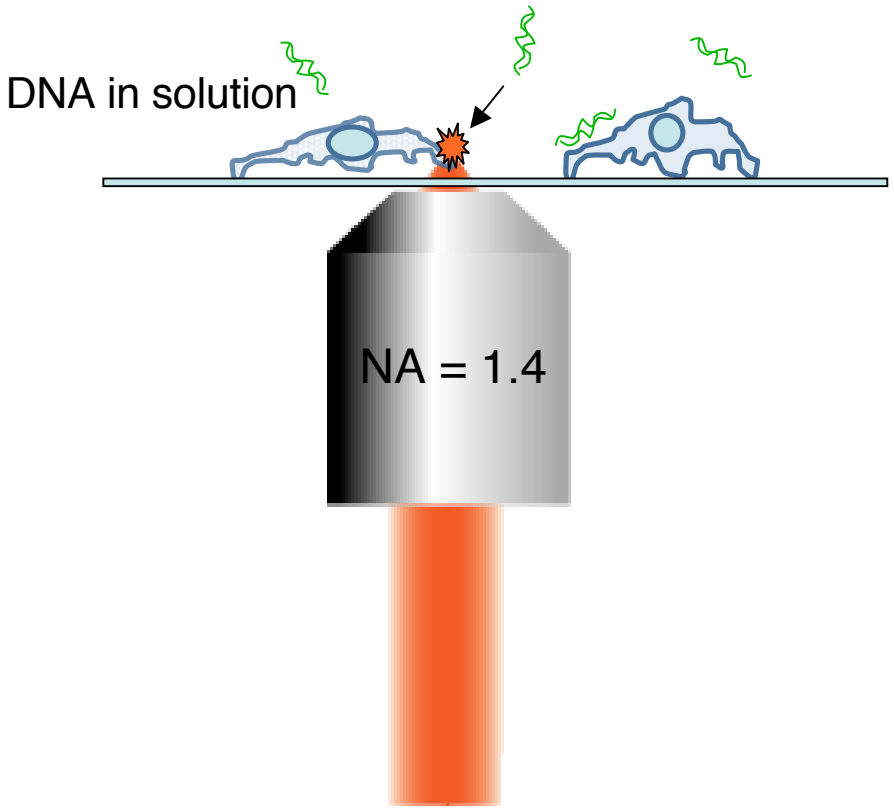
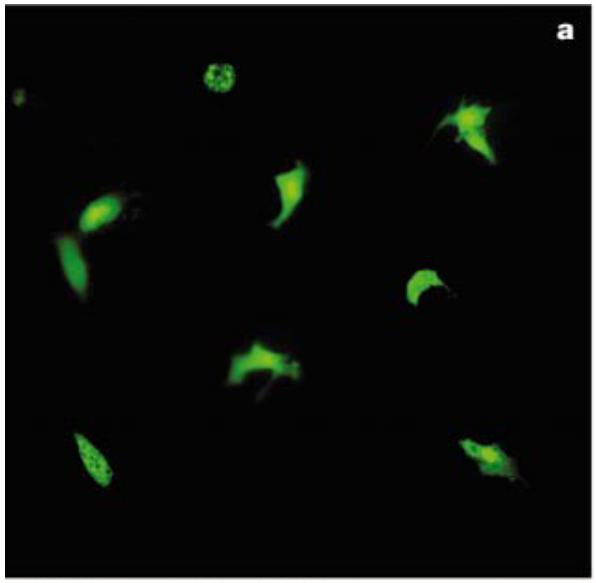
Linear vs. nonlinear absorption



transparent material

Background: femtosecond laser cell transfection



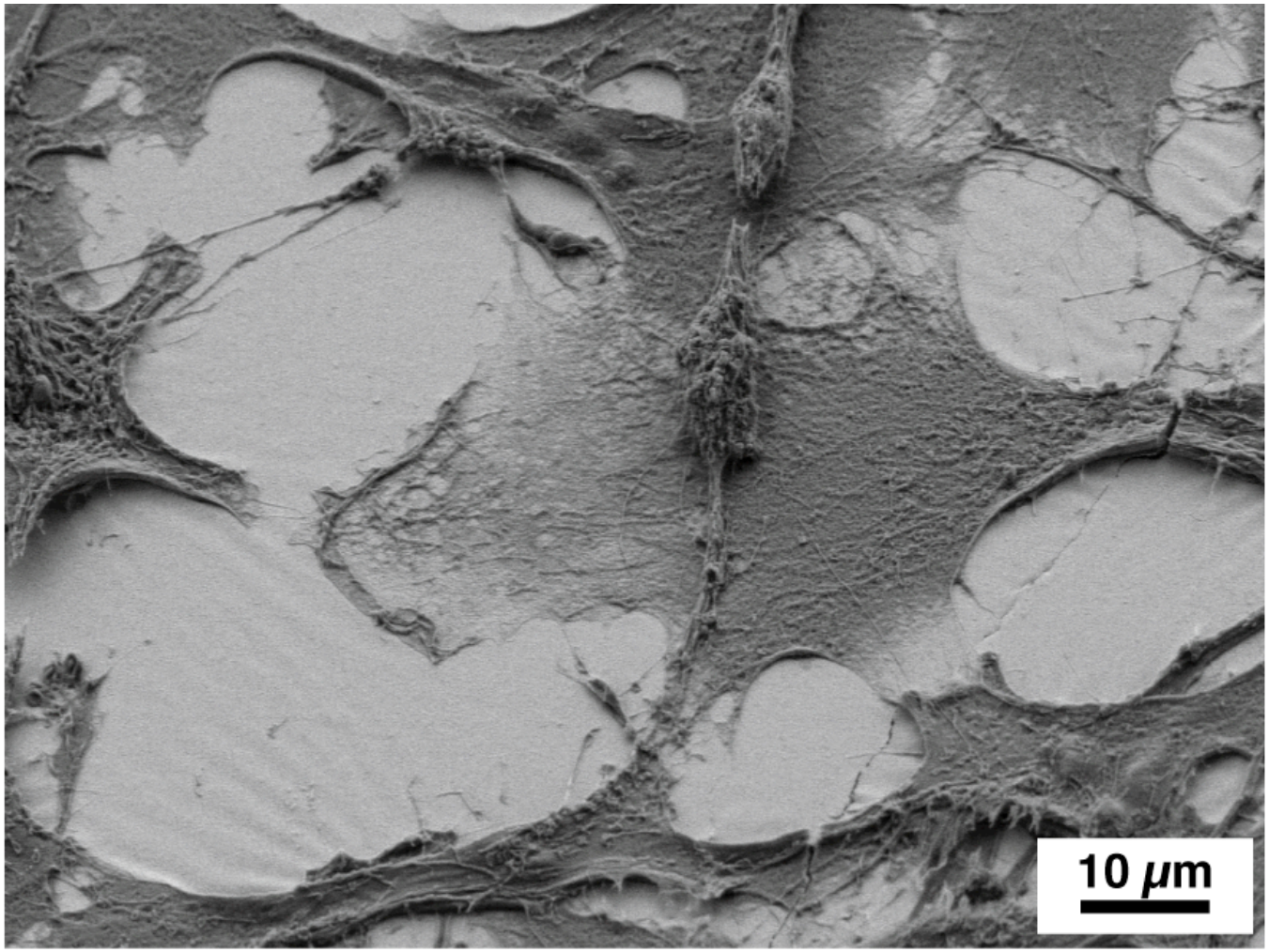


Transfection of cells
with near-100% efficiency

Excellent efficiency, but terrible
throughput!

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

Background: femtosecond laser cell transfection



Human CD4 thymic epithelial cells, on flat substrate

Background: femtosecond laser cell transfection

How do we optimize the laser parameters?

Laser parameters are critical to cell viability:

High-NA (>1.0) focusing
50-200mW avg. power at 80 MHz repetition rate
~100 fs pulse duration
10-100 ms exposure time

Background: femtosecond laser cell transfection

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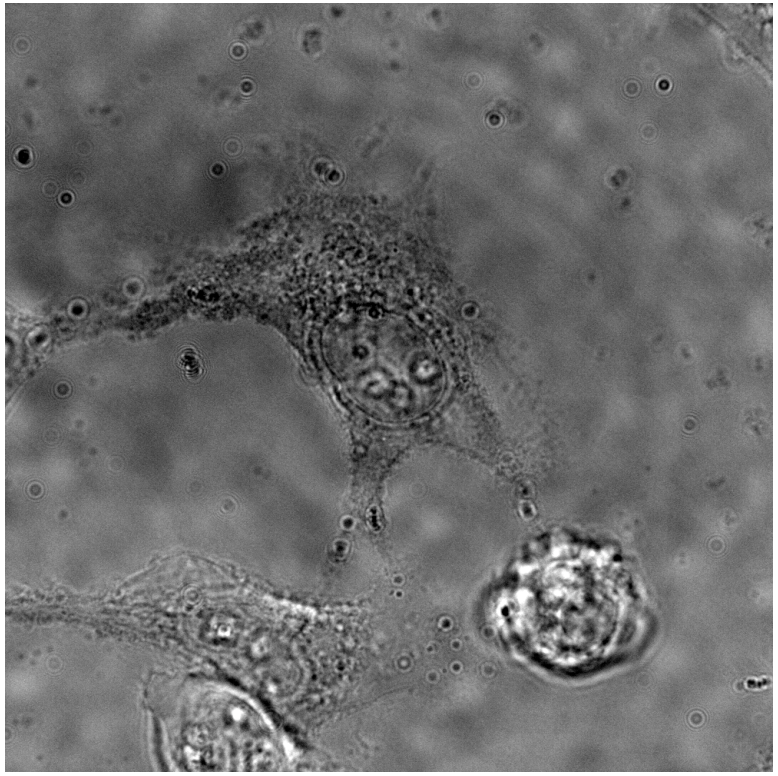
Pore size smaller than ~2μm is required for cell viability

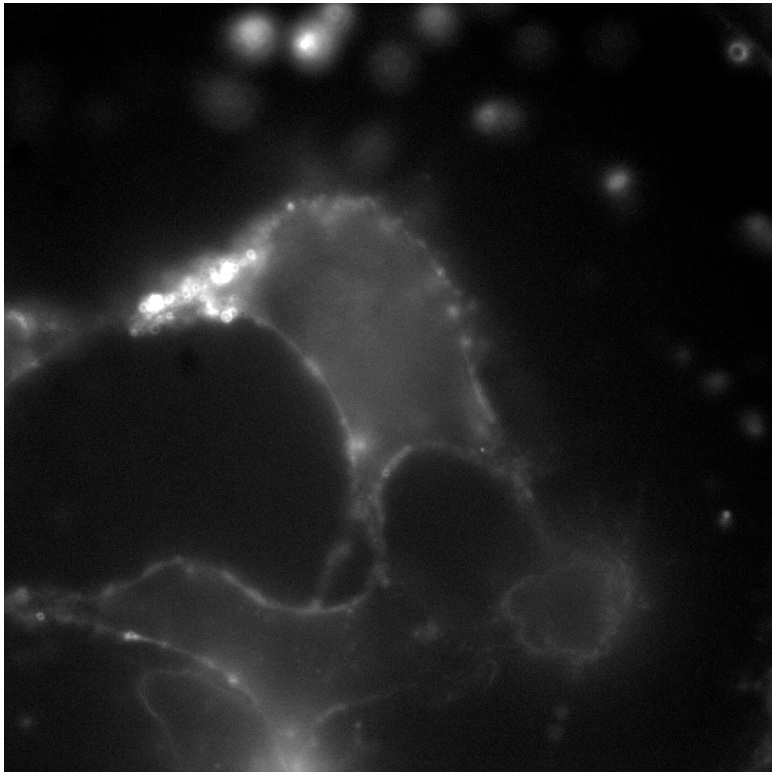
Background: femtosecond laser cell transfection

How do we optimize the laser parameters?

Using a lipid-sensitive fluorescent dye, we can monitor diffusion into the cell, as well as cell wound-healing.



brightfield



fluorescence (488-nm excitation)

Outline

SERS hot spot isolation:

Background: laser nanostructured substrates

Motivation: hot spot distribution

Hot spot isolation

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Ultrafast plasmon-cell interactions

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

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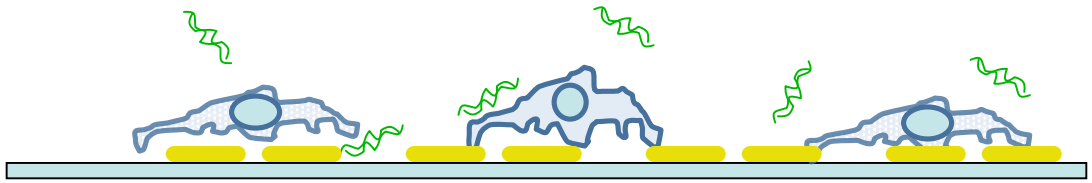

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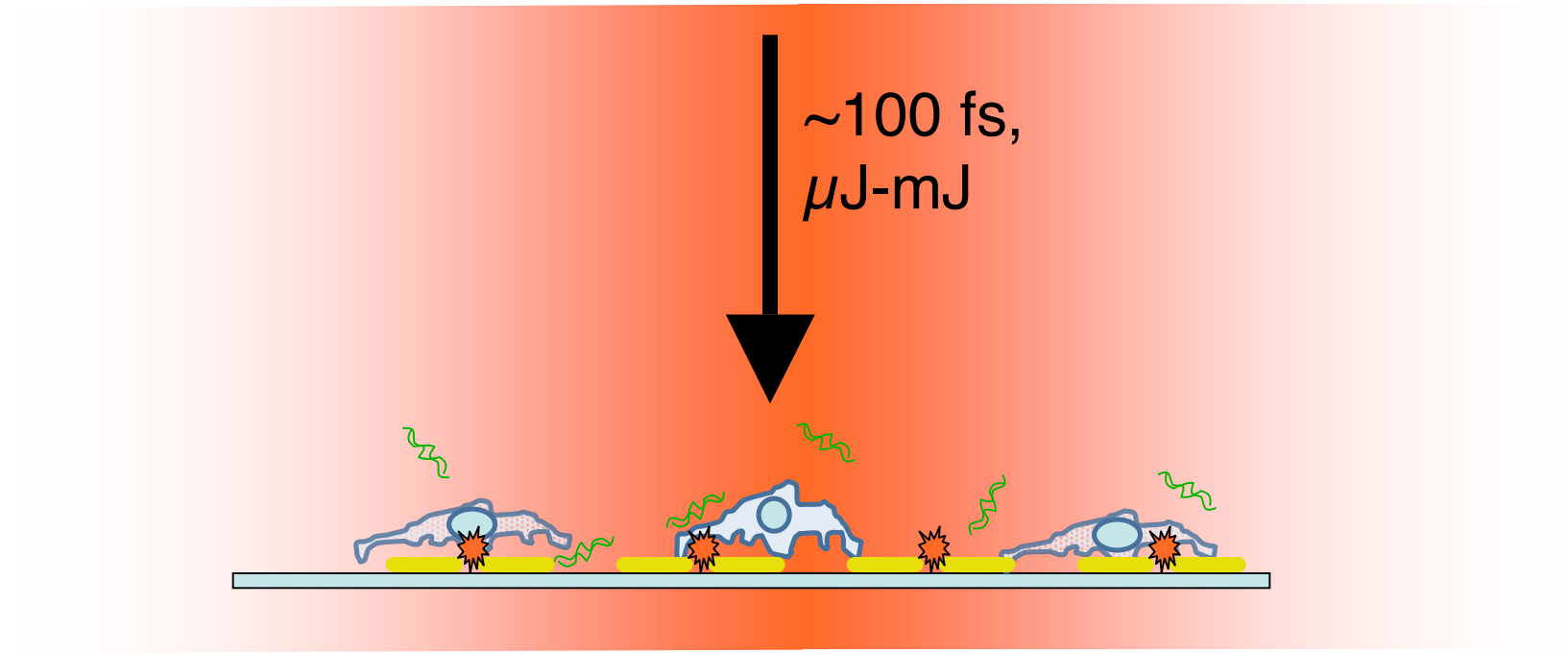

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Ultrafast plasmon-cell interactions

The choice of plasmonic substrate has specific requirements:

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

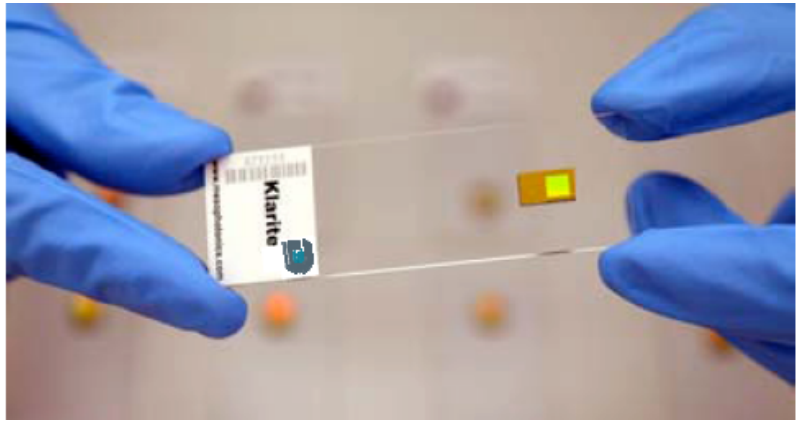
2. Field enhancing regions (areas of damage) must be accessible to DNA, RNA, etc. in surrounding solution.

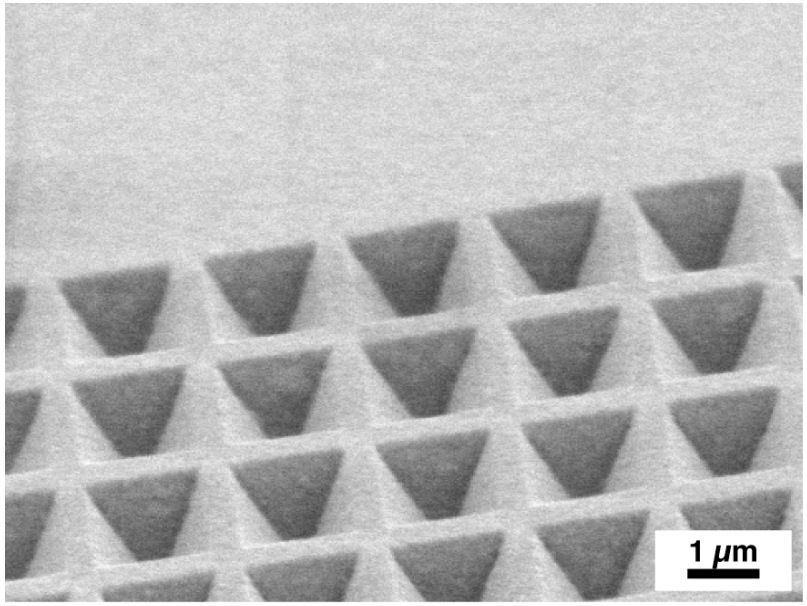
3. Substrate fabrication method must be scalable in size.

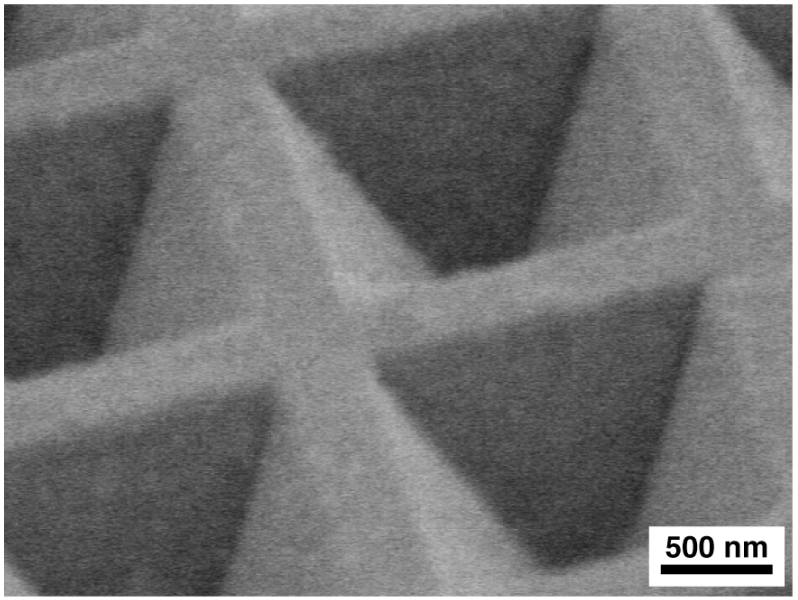
Ultrafast plasmon-cell interactions

Proposed substrate: template-stripped gold pyramid array

KOH-etched silicon wafer:
Gold coated, supports NIR localized plasmons inside pyramidal pits



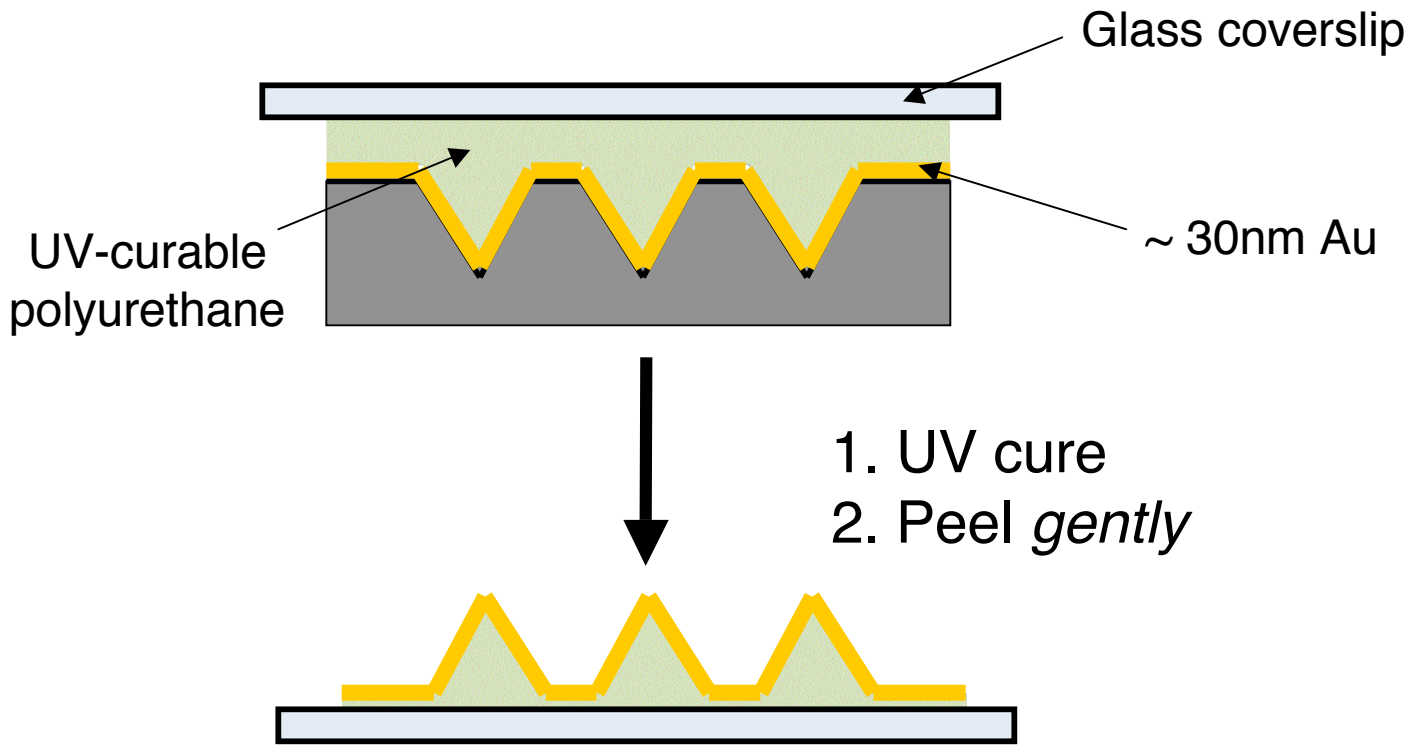




Ultrafast plasmon-cell interactions

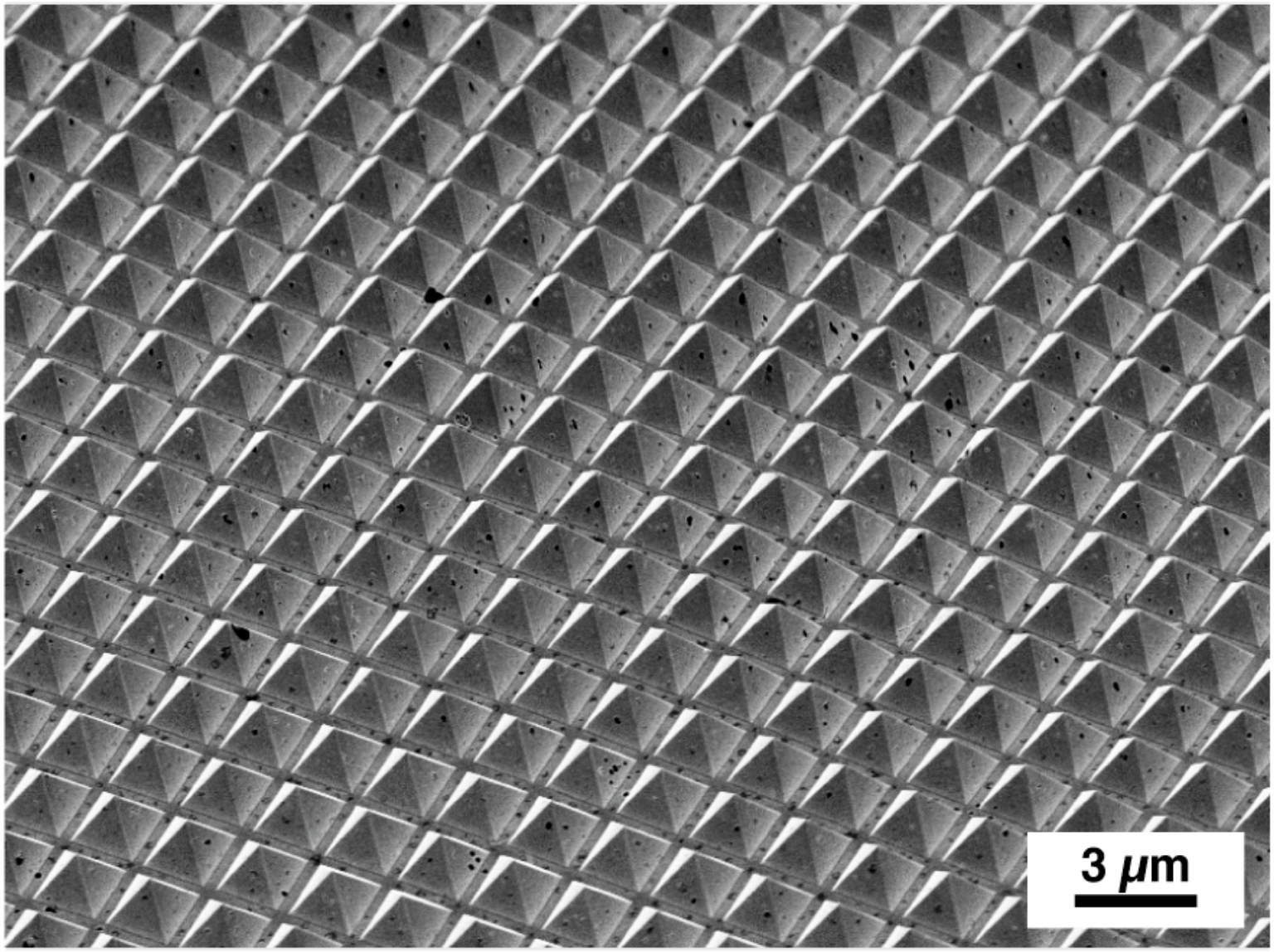
Proposed substrate: template-stripped gold pyramid array

Template stripping exploit poor adhesion of noble metals on silicon



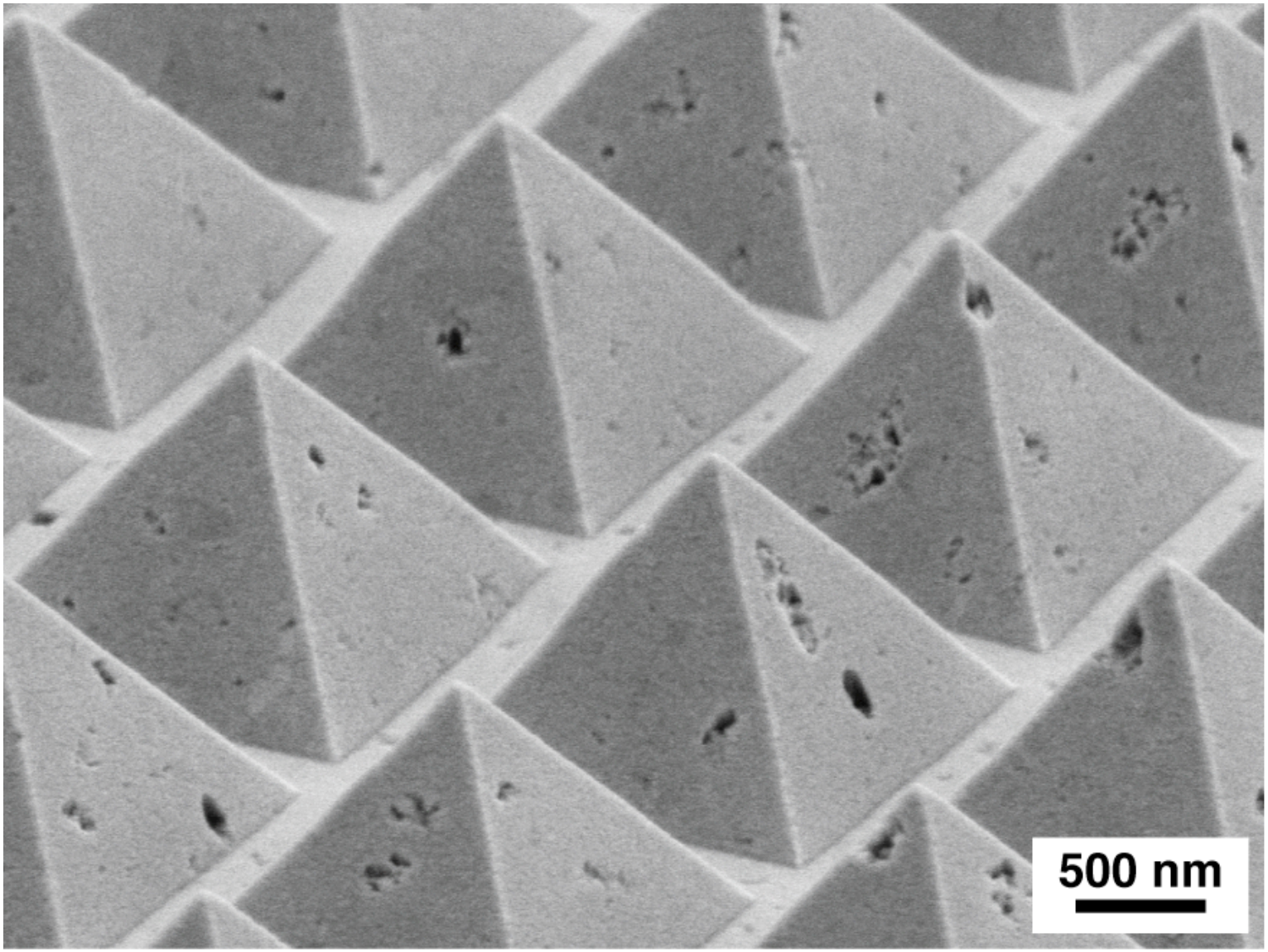
P. Nagpal et al. *Science* **325**, 594 (2009)

Ultrafast plasmon-cell interactions



30nm Au on polyurethane

Ultrafast plasmon-cell interactions



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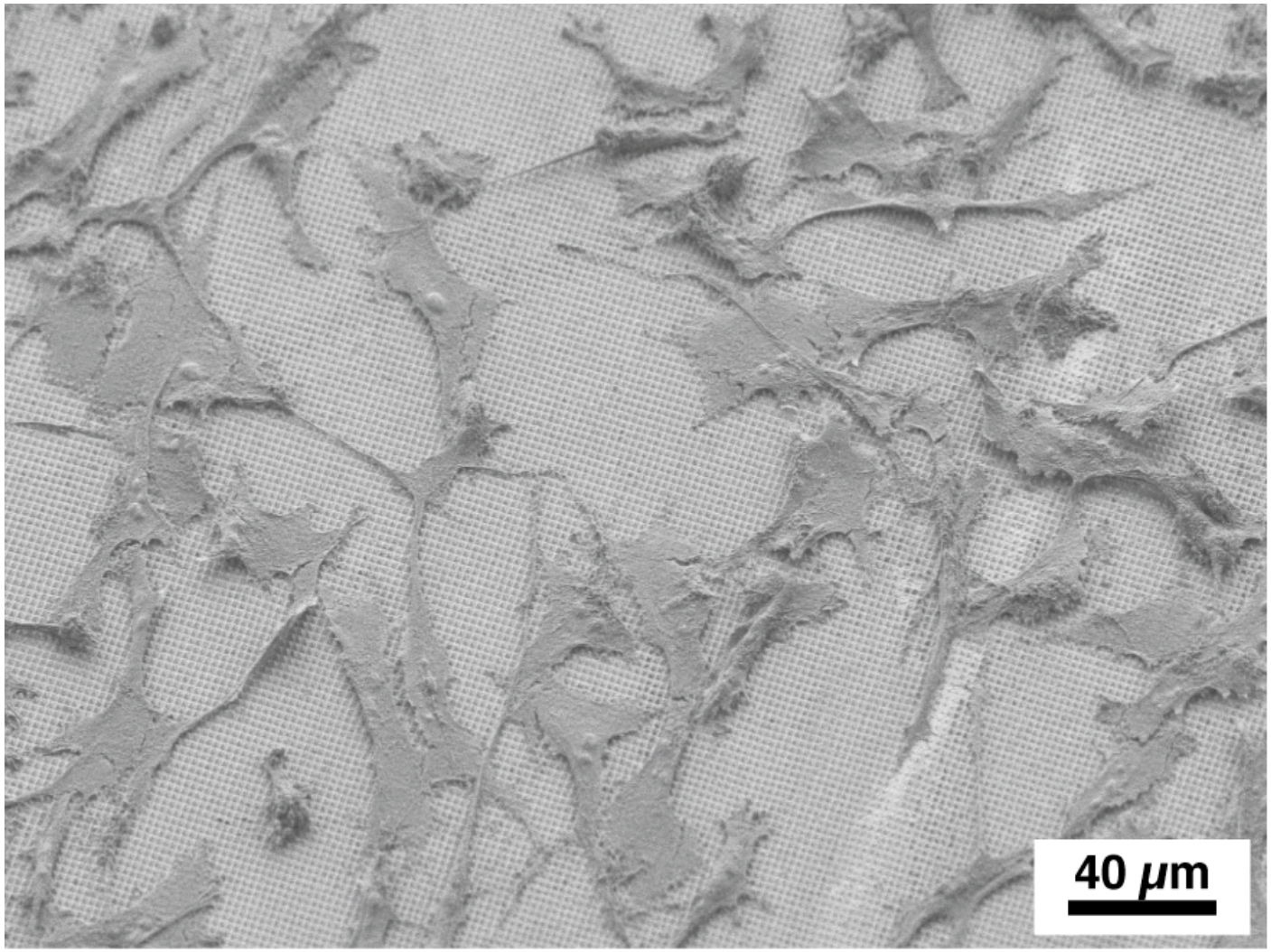
Ultrafast plasmon-cell interactions

Cell viability on pyramidal substrates



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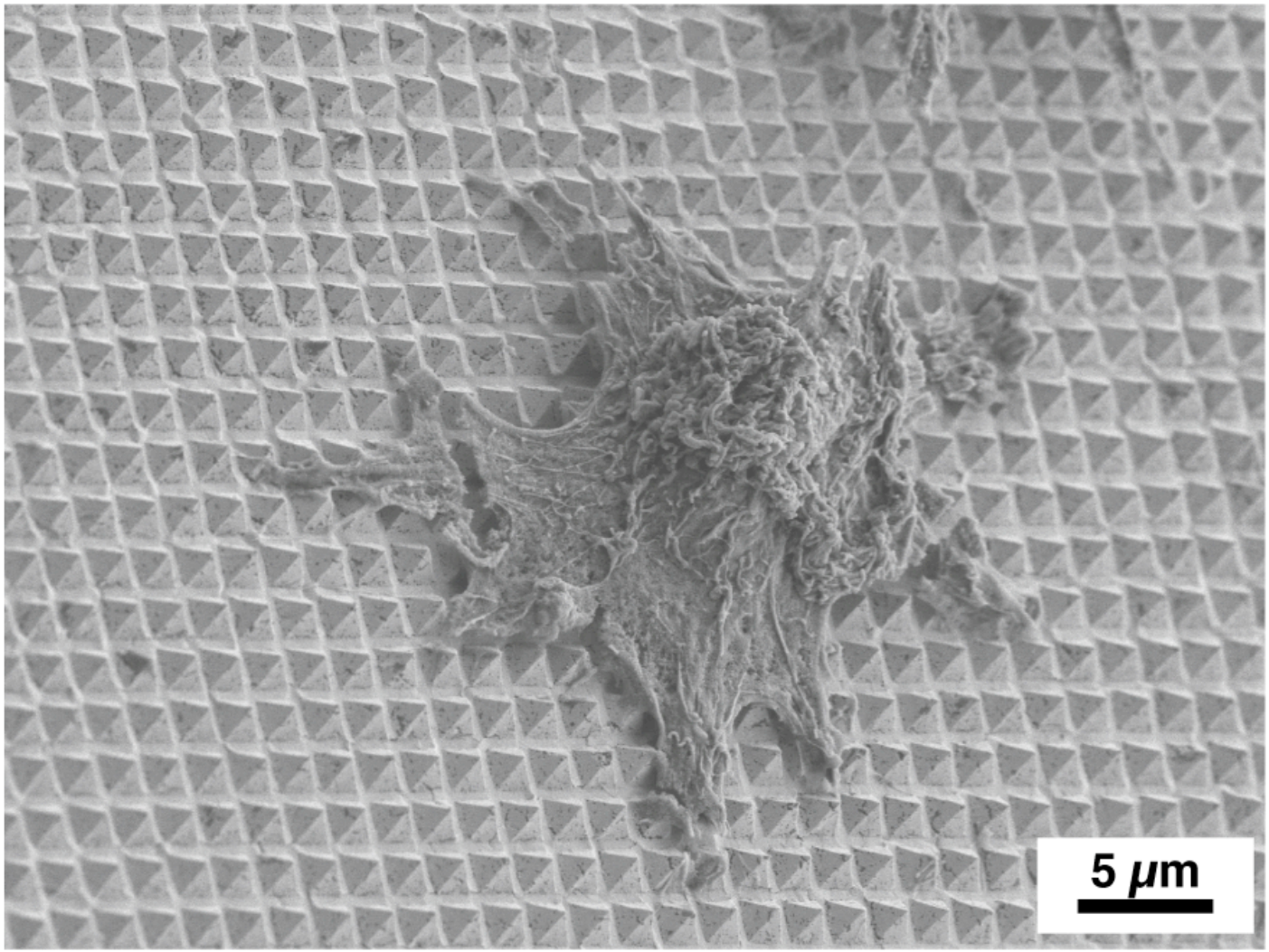
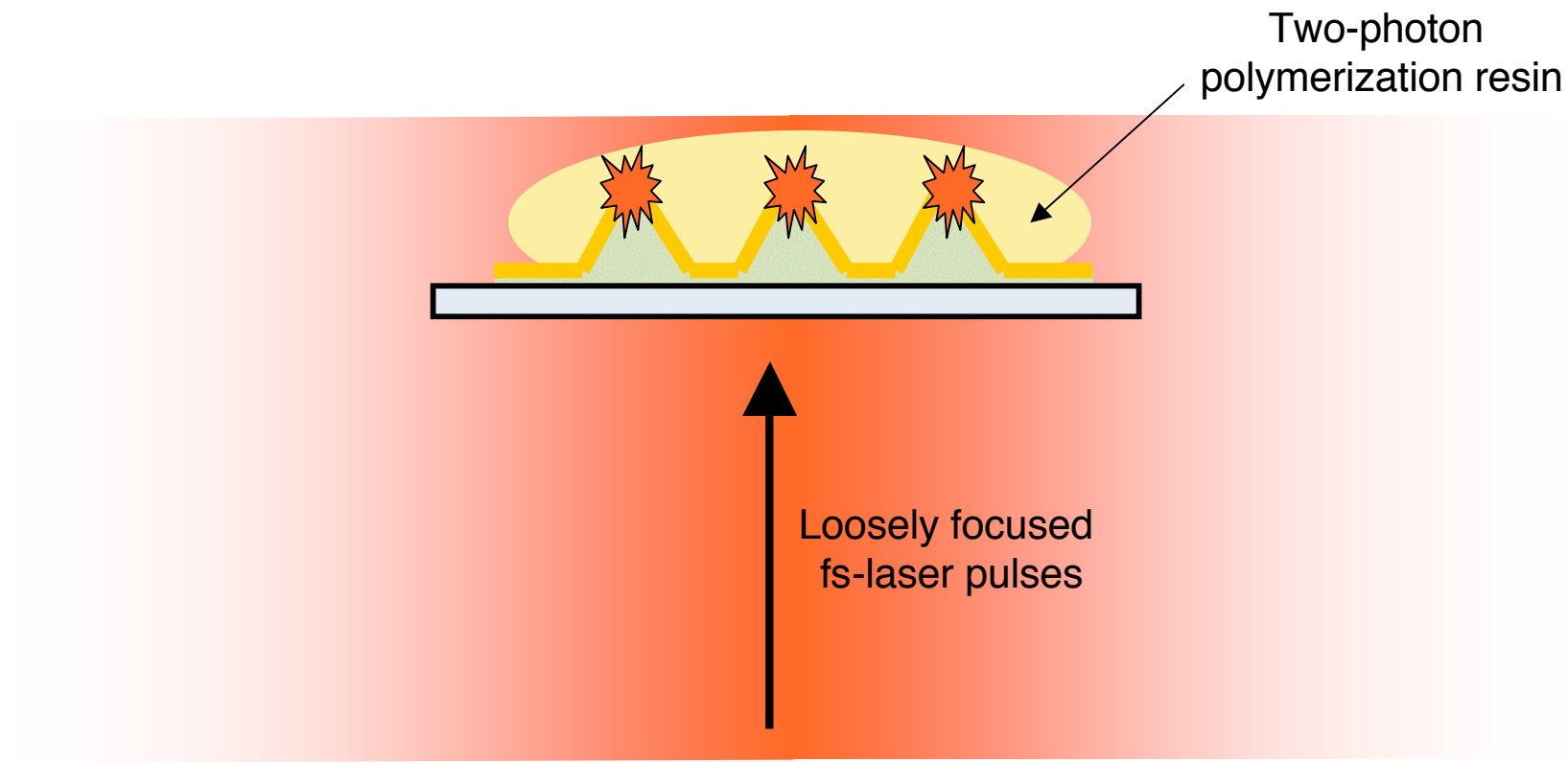


Where is the field enhancement?

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



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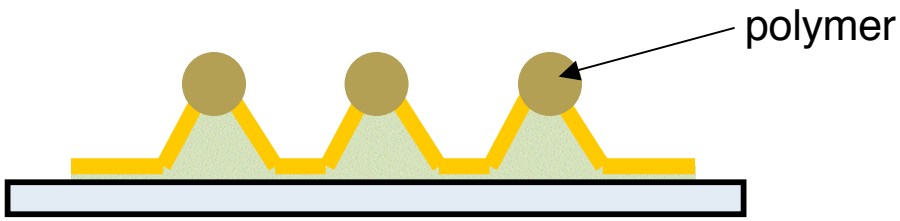

after washing in ethanol

Where is the field enhancement?

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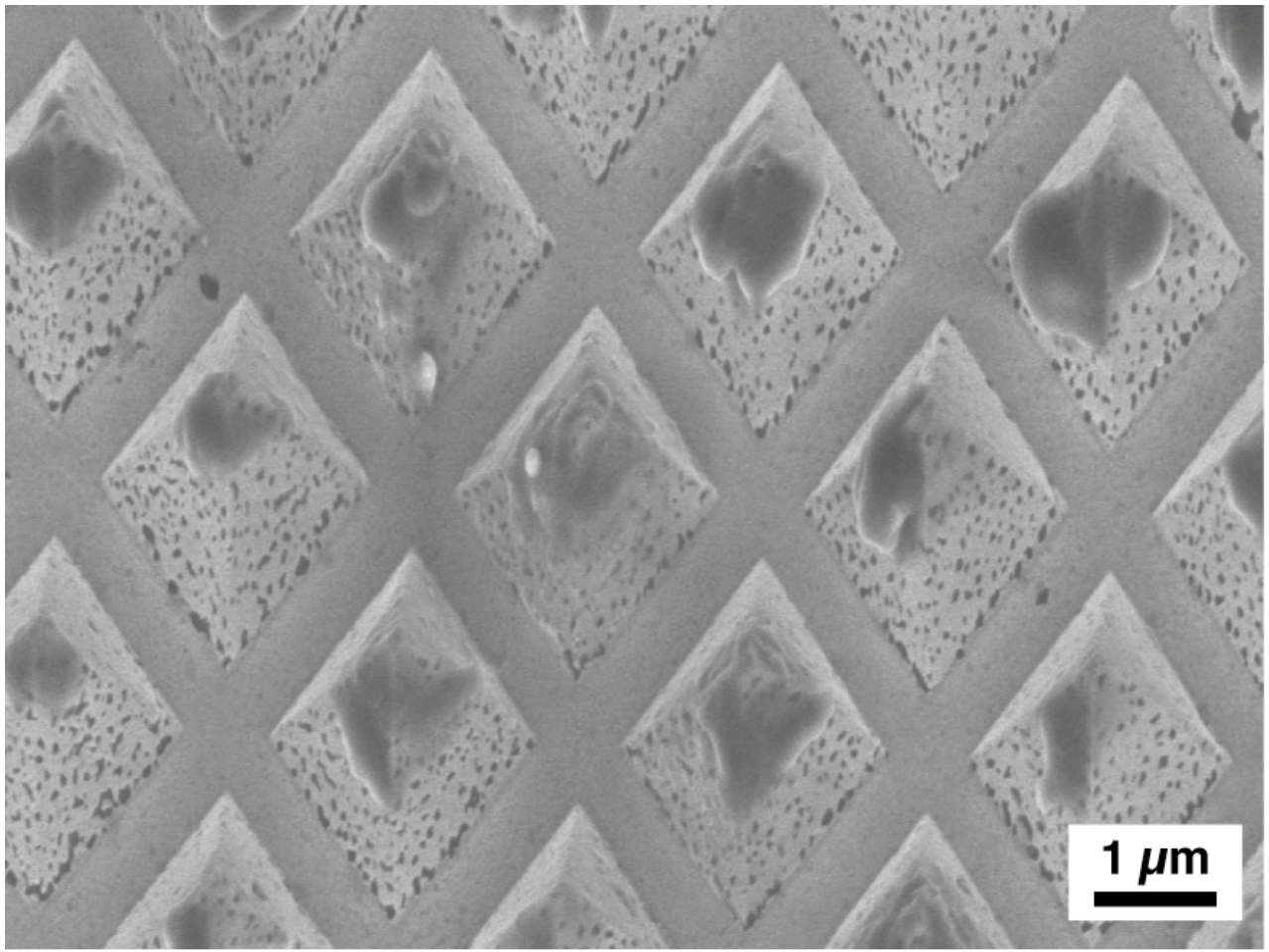


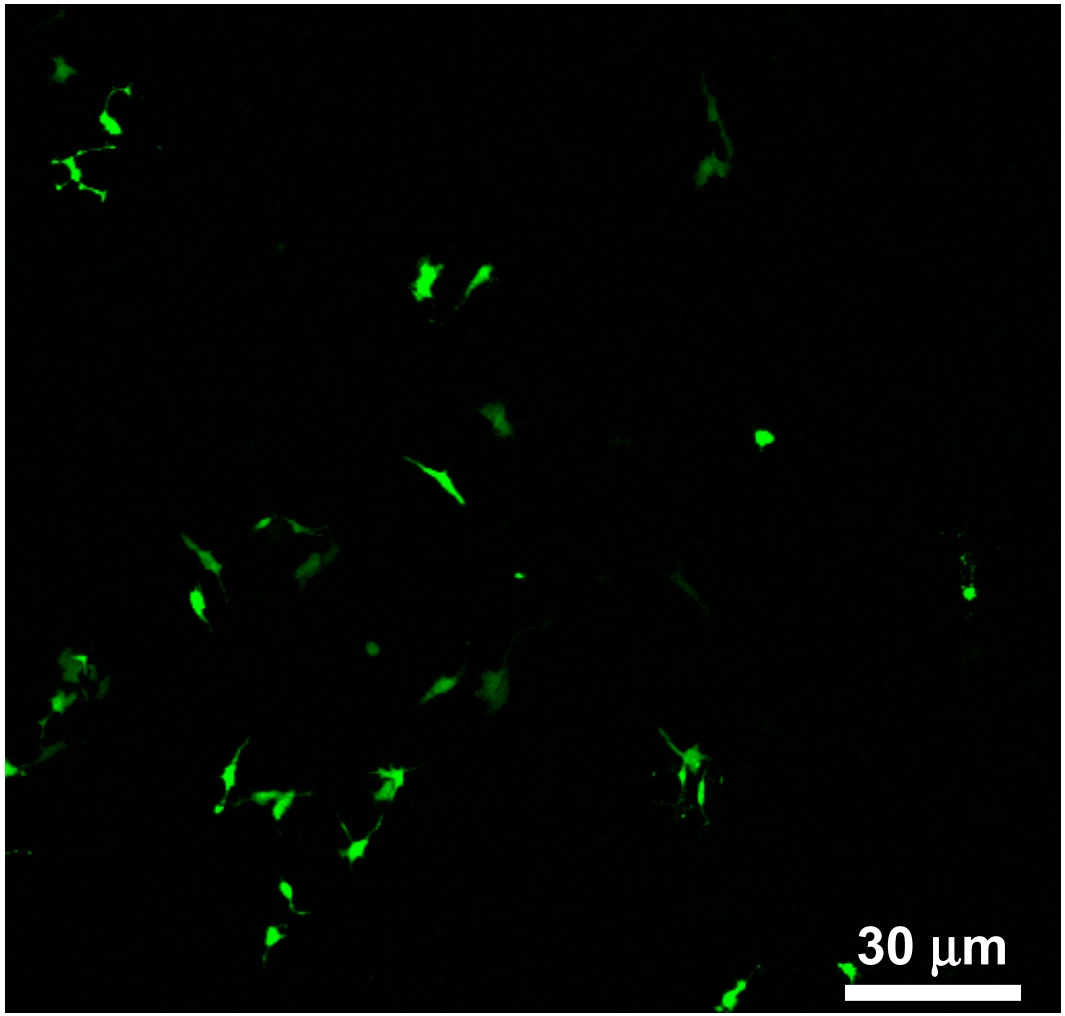
Plasmon-enhanced cell perforation

Using the pyramidal substrate, we observe a 32x reduction in pulse energy to generate cell perforation comparable to flat coverslip substrate.

Ultrafast plasmon-cell interactions

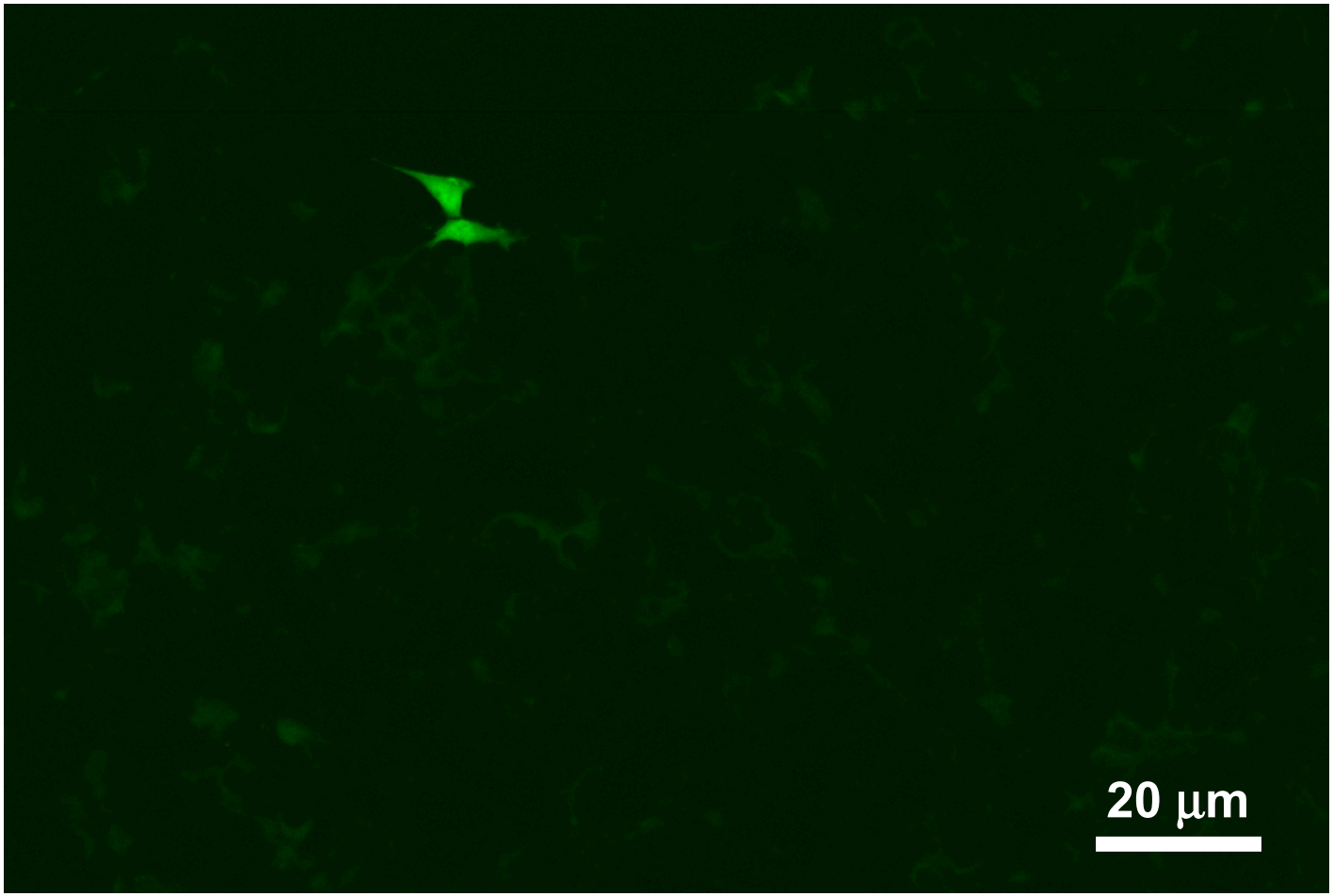
Green fluorescent protein DNA transfection:

State of the art, lipid-based reagents



Ultrafast plasmon-cell interactions

Green fluorescent protein DNA transfection:
Pyramidal substrate, large-area laser scan



Take home message

1. Large-area plasmonic substrates are bio-compatible for use with large numbers of cells.

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1. Large-area plasmonic substrates are bio-compatible for use with large numbers of cells.

2. Plasmonic substrates can reduce laser energy threshold for localized cell membrane damage.

3. Specialized design requirements (hot spot pitch, location, aspect ratio) are important for plasmon-enhanced transfection.

Thank you!

Mazur group

Dr. Andrew Koh (HMS, now Stanford)

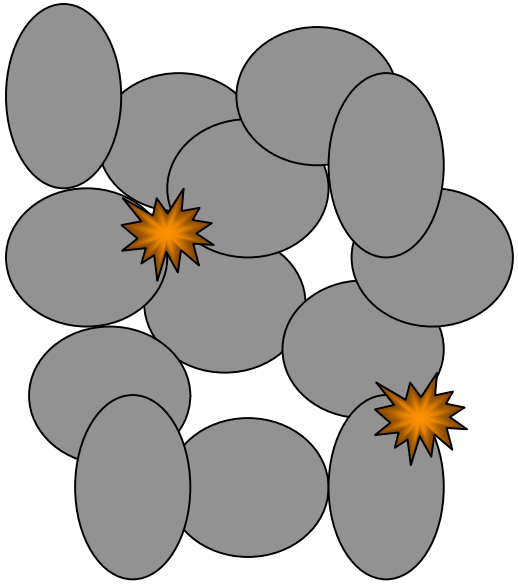
Center for Nanoscale Systems, Harvard University

DARPA: SERS S&T Fundamentals Program

Hot spot isolation

Hot spots in random metallic nanoparticle clusters exhibit large spatial dispersion.

785 nm



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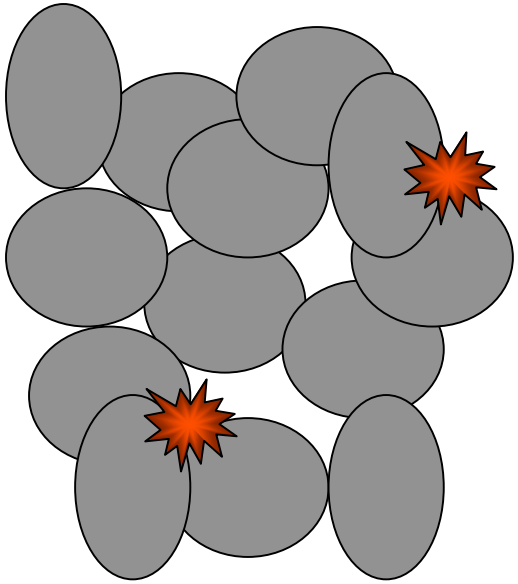
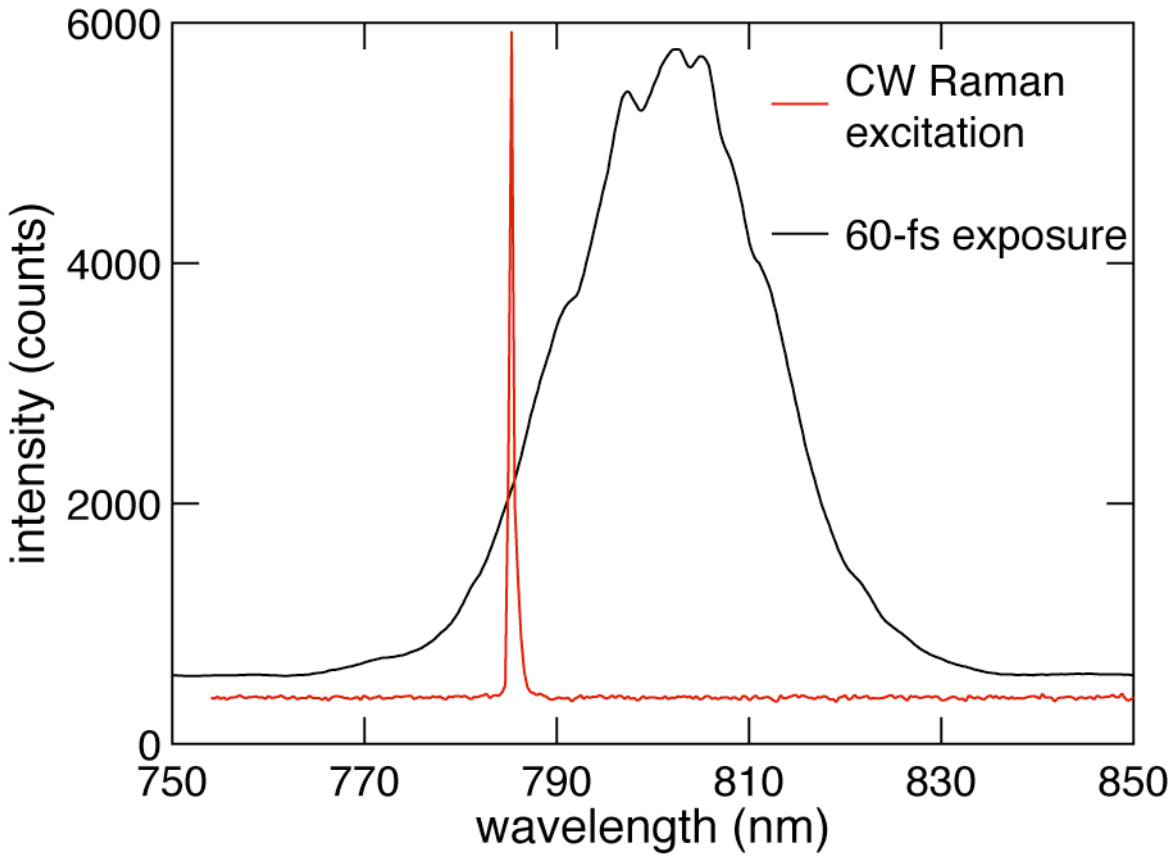


Grésillon et al. *Phys. Rev. Lett.* **82** 4520-4523 (1999)

Hot spot isolation



Hot spot dispersion necessitates overlap of Raman excitation and fs-exposure spectra.