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

Eric Diebold
Harvard University

Brongersma group seminar 1/26/10
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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Raman scattering

\[
\begin{align*}
\text{Energy} & \\
\text{Electronic states} & \\
\text{Virtual states} & \\
\text{Vibrational states} & \\
\text{Ground state} &
\end{align*}
\]
Background

Raman scattering

- Electronic states
- Virtual states
- Vibrational states
- Ground state

Energy

Stokes
Raman
Raman scattering

- **Ground state**
- **Vibrational states**
- **Virtual states**
- **Electronic states**

Energy scale:
- **Stokes**
- **Raman**
Raman scattering

- Electronic states
- Virtual states
- Vibrational states
- Ground state

Stokes Raman
Anti-Stokes Raman
Raman scattering

$$\Delta \nu = \frac{1}{\lambda_{\text{incident}}} - \frac{1}{\lambda_{\text{scattered}}}$$
Background

\[ a < 0.05 \lambda \]
1. Near-field scattered electric field enhances polarization of molecules located near surface

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

3. Sphere polarization radiates Raman field into far field
Background

Electromagnetic SERS enhancement factor:

\[
\frac{I_{SERS}}{I_{\text{Normal Raman}}} \propto \left( \left| \frac{E_s(\omega_0)}{E_0(\omega_0)} \right| \right)^2 \times \left( \left| \frac{E_s(\omega_0 - \omega_R)}{E_0(\omega_0 - \omega_R)} \right| \right)^2 \approx \left( \left| \frac{E_s(\omega_0)}{E_0(\omega_0)} \right| \right)^4
\]
Electromagnetic SERS enhancement factor:

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

\[
\sigma' \approx \sigma \left| \frac{\varepsilon_1(\omega) - \varepsilon_2}{\varepsilon_1(\omega) + 2\varepsilon_2} \right|^2 \left| \frac{\varepsilon_1(\omega) - \varepsilon_2}{\varepsilon_1(\omega) + 2\varepsilon_2} \right|^2 \left( \frac{a}{a + r} \right)^{12}
\]
Background
Background
Background

Background

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

Average enhancement factor (benzenethiol) \( \sim 10^7 \)

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Motivation: plasmonic substrates

Ultrafast plasmon-cell interactions
Motivation: hot spot distribution

Measurement of the Distribution of Site Enhancements in Surface-Enhanced Raman Scattering
Ying Fang, Nak-Hyun Seong, Dana D. Dlott

<table>
<thead>
<tr>
<th>Raman enhancement factor $\eta$</th>
<th>Percentage of molecules</th>
<th>Percentage contribution to overall SERS signal</th>
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<tbody>
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<td>$&lt;2.8 \times 10^4$</td>
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<tr>
<td>$2.8 \times 10^4$ to $1 \times 10^5$</td>
<td>61%</td>
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Motivation: hot spot distribution

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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%!

Motivation: hot spot distribution

If $N_{\text{analyte}}$ is small, how do we ensure that molecules adsorb only to hot spots?
Motivation: hot spot distribution

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Mask

“cold” spots (covered)

“hot” spot (uncovered)
Motivation: hot spot distribution

- Mask
- “cold” spots (covered)
- “hot” spot (uncovered)
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SERS hot spot isolation:
Background: laser nanostructured substrates
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Hot spot isolation

Plasmon-enhanced laser cell transfection:
Background: femtosecond laser cell transfection
Motivation: plasmonic substrates

Ultrafast plasmon-cell interactions
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

Hot spot isolation

HSI substrates expected to show higher enhancement

*under conditions of sub-monolayer coverage.*

\[ N_{\text{analyte}} \ll N_{\text{adsorption sites}} \]
Hot spot isolation

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

\[ \text{N}_{\text{analyte}} \ll \text{N}_{\text{adsorption sites}} \]

HSI-SERS substrate

- Analyte binds exclusively to exposed hot spots

SERS substrate

- Analyte distributed over both hot and cold spots
HSI substrates expected to show higher enhancement under conditions of sub-monolayer coverage.

\[ N_{\text{analyte}} \ll N_{\text{adsorption sites}} \]

HSI-SERS substrate

Analyte binds exclusively to exposed hot spots

SERS substrate

Analyte distributed over both hot and cold spots

Benzenethiol
Hot spot isolation

$\lambda_{\text{center}} = 795\text{nm}$, $\tau = 60\text{fs}$, 100 pulses/spot
Hot spot isolation

\[ \lambda_{\text{center}} = 795\text{nm}, \; \tau = 60\text{fs}, \; 100 \text{ pulses/spot} \]
Hot spot isolation
Hot spot isolation
Hot spot isolation
Hot spot isolation
Hot spot isolation

Increasing fluence
Hot spot isolation

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

Hot spot isolation

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

Submonolayer coverage:
  24 hour incubation with $2.4 \times 10^9$ molecules = 0.001% surface coverage.
Hot spot isolation

Average enhancement factor:

Submonolayer coverage:
24 hour incubation with $2.4 \times 10^9$ molecules = 0.001% surface coverage.

Signal normalized to neat benzenethiol using confocal microscope method.

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

![Diagram showing the setup with a hot spot isolation technique involving a coverslip, glass slide, silicone spacer, and neat benzenethiol.](diagram.png)
Hot spot isolation

Average enhancement factor:

Submonolayer coverage:
24 hour incubation with $2.4 \times 10^9$ molecules = 0.001% surface coverage.

Signal normalized to neat benzenethiol using confocal microscope method.

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

Enhancement factor (998 cm$^{-1}$) = $3 \times 10^9$
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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2. does not require knowledge of hot spot location or enhancement factor.

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.

Take home message
Plasmon-enhanced laser cell transfection
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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
Cell transfection is central to:

Genetic engineering

Potential gene therapies - DNA, siRNA, etc.

Basic biological research
Background: femtosecond laser cell transfection

Background: femtosecond laser cell transfection

Background: femtosecond laser cell transfection

Linear vs. nonlinear absorption

\[ N_{abs} \propto \sigma_1 I^1 \]

\[ N_{abs} \propto \sigma_n I^n \]

\( E_c \)

\( E_v \)

\( n \) photons
Background: femtosecond laser cell transfection

Linear vs. nonlinear absorption

\[ N_{\text{abs}} \propto \sigma_1 I^1 \]
\[ N_{\text{abs}} \propto \sigma_n I^n \]

100 fs

Transparent material
Background: femtosecond laser cell transfection

Linear vs. nonlinear absorption

\[ N_{abs} \propto \sigma_1 I^1 \quad \text{and} \quad N_{abs} \propto \sigma_n I^n \]

transparent material
Background: femtosecond laser cell transfection

DNA in solution

800nm, 100fs, 1nJ, 80MHz

Transfection of cells with near-100% efficiency

Excellent efficiency, but terrible throughput!

Background: femtosecond laser cell transfection

Human CD4 thymic epithelial cells, on flat substrate
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
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

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](image1.jpg)  ![fluorescence (488-nm excitation)](image2.jpg)
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Use sub-wavelength focusing of plasmonic nanostructures to replace high-NA focusing.
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Larger laser pulse energies combined with larger spot sizes and/or scanning, many cells can be transfected quickly.
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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

1. UV cure
2. Peel gently

Ultrafast plasmon-cell interactions

30nm Au on polyurethane
Ultrafast plasmon-cell interactions

30nm Au on polyurethane
Ultrafast plasmon-cell interactions

Cell viability on pyramidal substrates
Ultrafast plasmon-cell interactions

Cell viability on pyramidal substrates
Ultrafast plasmon-cell interactions

Where is the field enhancement?

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

Where is the field enhancement?

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

after washing in ethanol
Ultrafast plasmon-cell interactions

Where is the field enhancement?

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

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

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

Take home message

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

Hot spot isolation

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