

Electroporation-introduced Gold Nanoparticles for Surface Enhanced Raman Spectroscopy of Biomolecules in Cells

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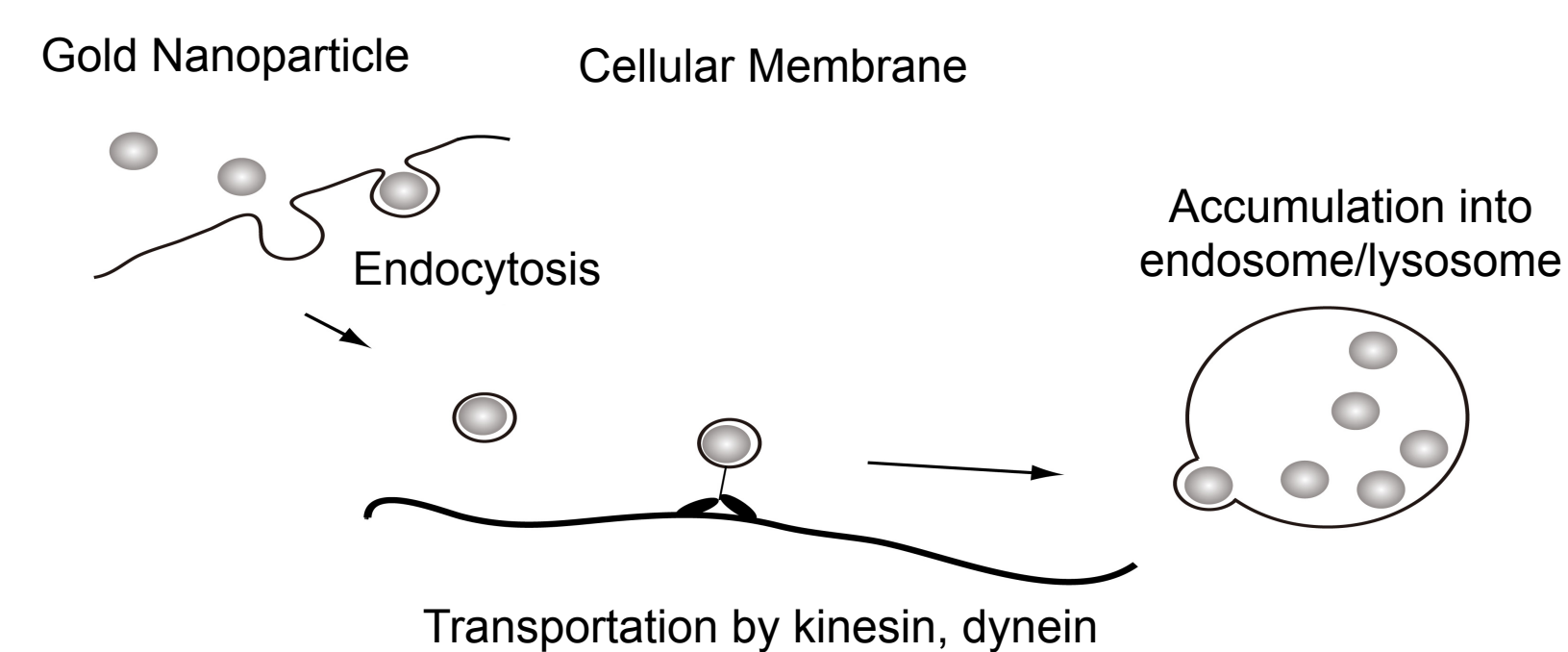
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Surface Enhanced Raman Spectroscopy (SERS) is a rapidly growing area of research which offers many applications in biomedical sciences. For example, this technique can be used to study molecular details of cellular processes or in disease diagnosis. SERS of biomolecules in cells relies on the introduction of metallic nanoparticles (NPs) which act as SERS probes. This is typically accomplished with passive cellular uptake through endocytosis. Endocytosis has several drawbacks, including a long incubation time for particle uptake and severe limitations on molecular information because particles are encased in lipid endosomes and are therefore not free in the cytoplasm. An alternative method for particle delivery, the creation of transient membrane pores through electroporation, was introduced recently and offers potential to solve these issues. Particle introduction with this method is faster than endocytosis, and NPs are expected to be free in the cytoplasm of the cell. We focus on the introduction of gold nanoparticles (AuNPs) into HeLa cells. Various parameters for electroporation were tested, and their effectiveness was ascertained by taking photoluminescence (PL) images with a 532 nm laser which causes strong PL of gold. PL method was chosen to allow for the location of AuNPs within cells to be determined despite the high lipid content of HeLa cells. We then performed SERS analysis, directly following PL imaging, with a slit-scanning Raman microscope (excitation wavelength 590 nm) on cells with particles introduced through electroporation and endocytosis to compare the spectral differences and similarities between the two methods of NP delivery.

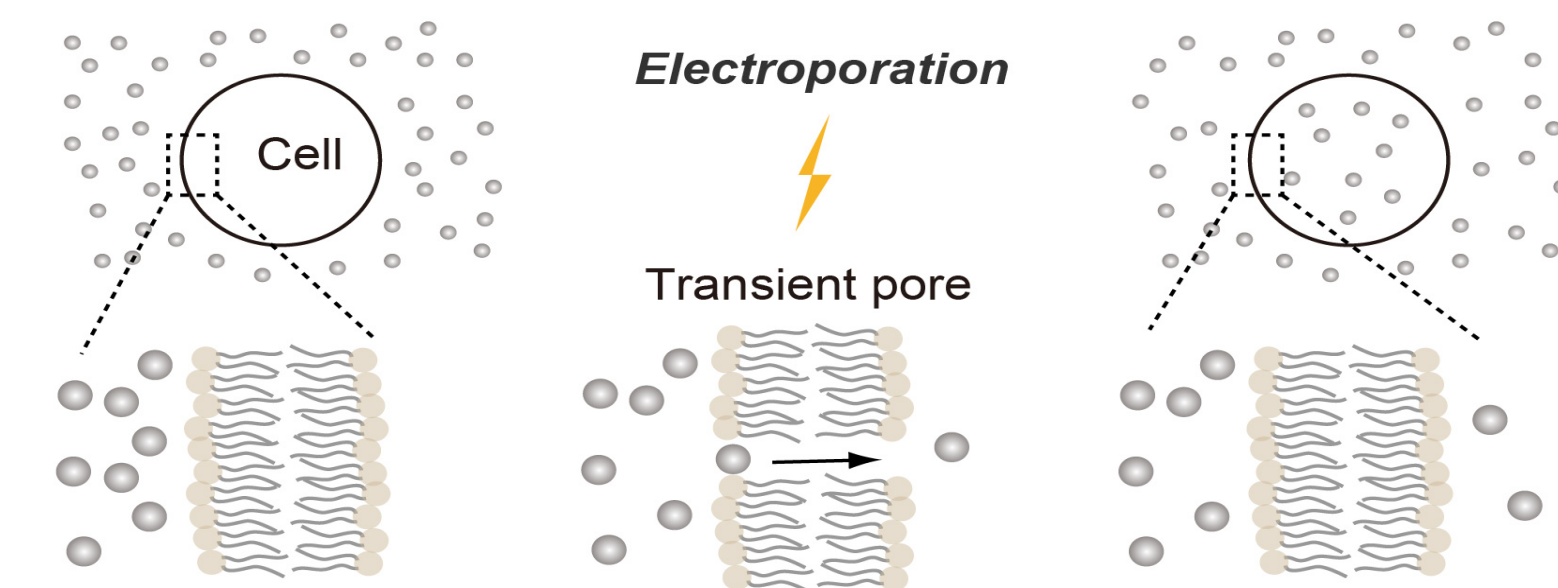
INTRODUCTION

Surface Enhanced Raman Spectroscopy (SERS) allows for highly-sensitive detection of biomolecules in cells using metal nanoparticles (NPs) to amplify Raman scattering signal in direct vicinity of NP surface

PROBLEM: Current method of NP delivery, **endocytosis**, is slow and limits information



Electroporation, introduced recently as new method, causes voltage-induced transient pores on cell membrane for direct delivery of NPs



GOALS:

- Determine suitable electroporation parameters to deliver AuNPs into HeLa cells
- Compare SERS information obtained from endocytosis- and electroporation- delivery

METHODS

Electroporation

- Suspended HeLa cells with 50 nm gold NPs
- Poring pulse: 575 V, 7.5 ms x 2, 10% decay
- Transfer pulse: 100 V, 50ms x 5, 40% decay

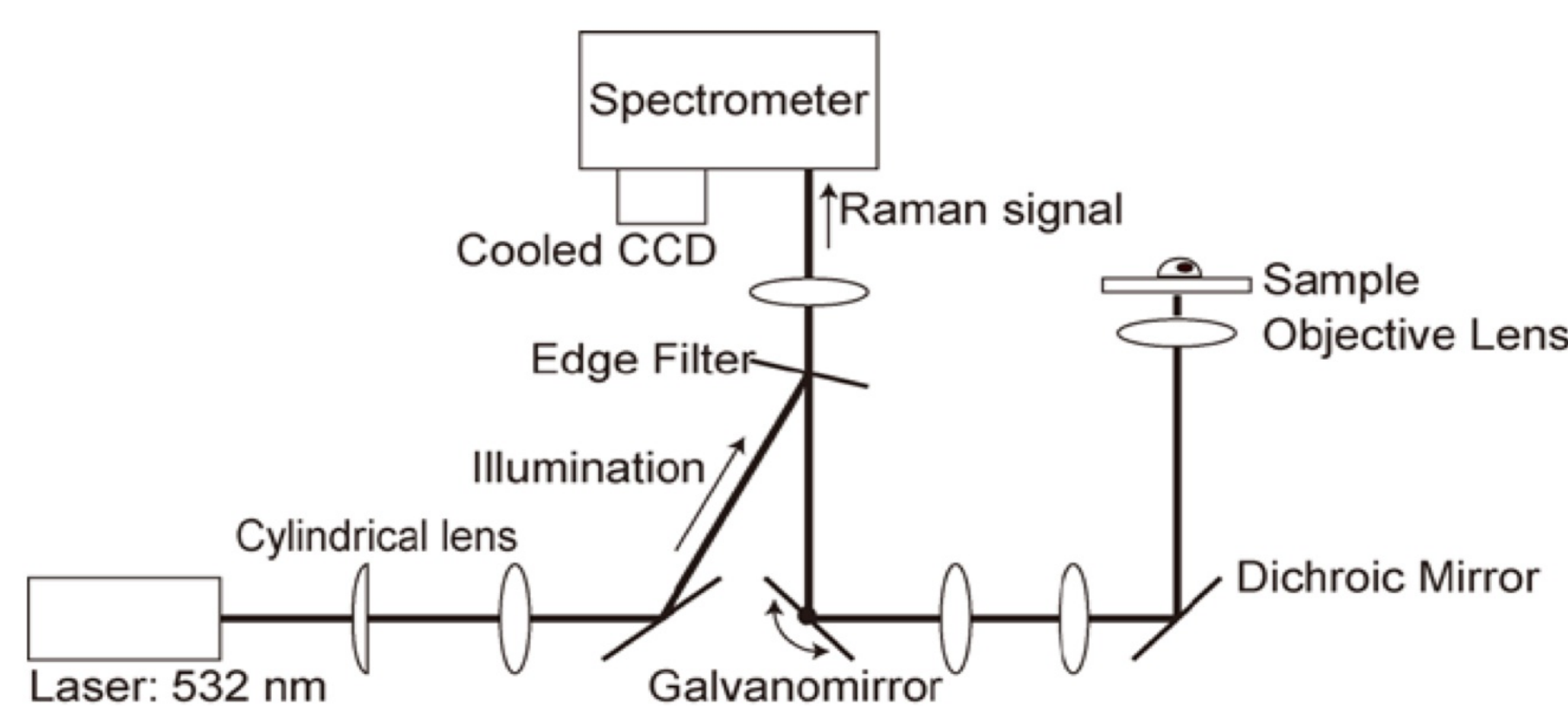
Photoluminescence (PL) imaging

- 532 nm excitation laser causes strong PL of gold
- Raman signal from CH₂ and CH₃ gives cell outline
- Allows distinction between particles and vesicles

SERS Imaging

- 590 nm excitation laser to obtain SERS signal

Optical layout of slit-scanning Raman microscope:



RESULTS

PL Images of Particles after Endocytosis

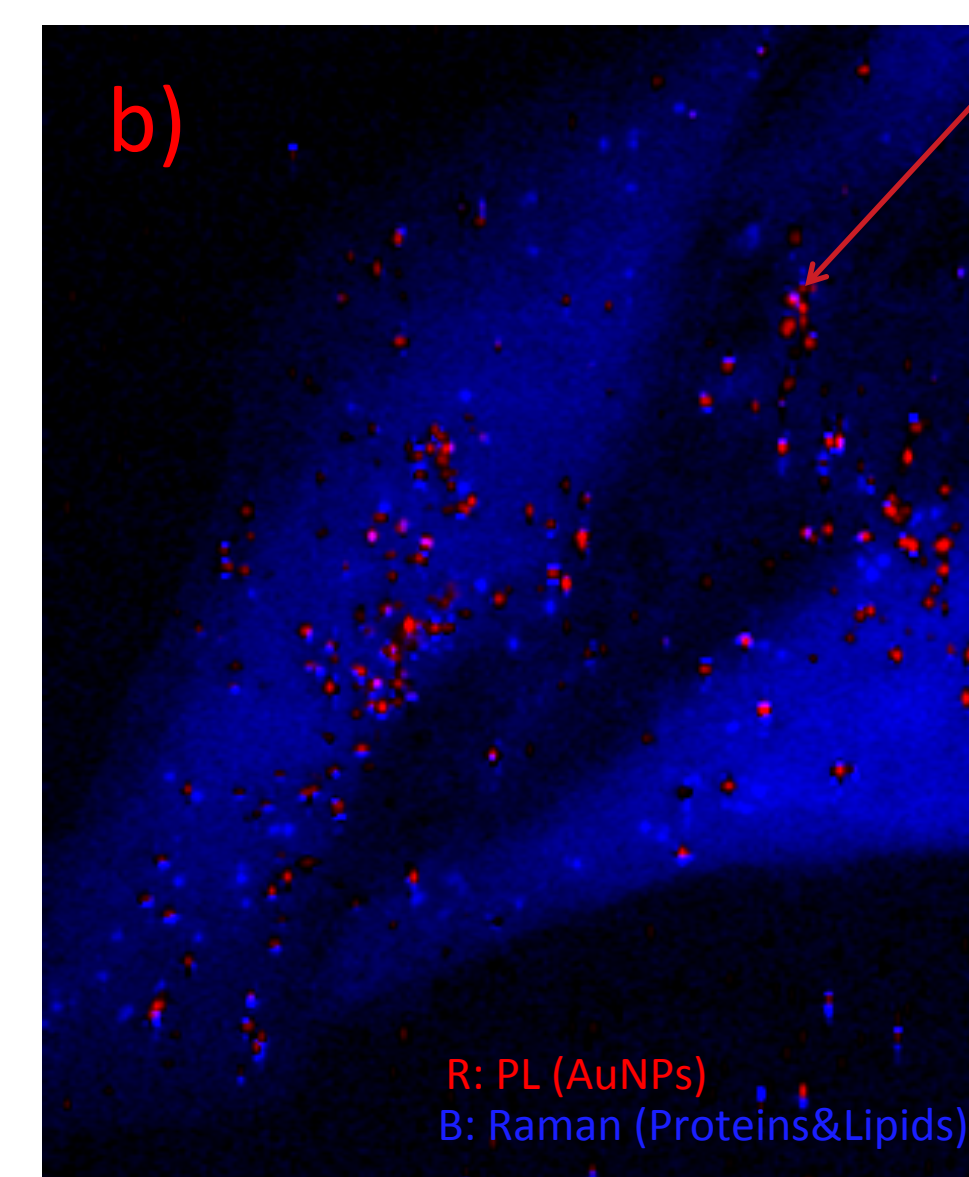
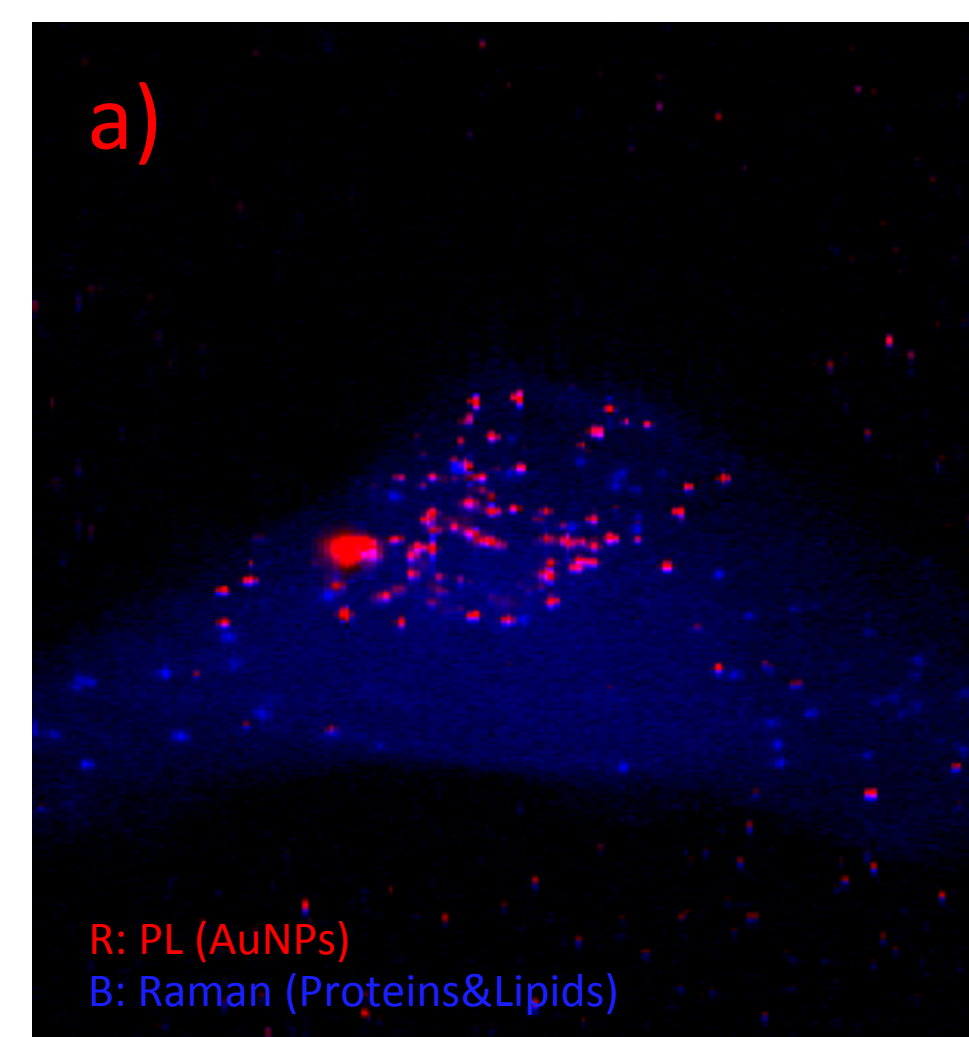


Fig. 1 (left): PL images of HeLa cells after uptake of (a) 20 nm AuNPs and (b) 50 nm AuNPs by endocytosis after 8 hours incubation. Red color shows PL hot spots from AuNPs, and blue color outlines cells. Excitation wavelength 532 nm.

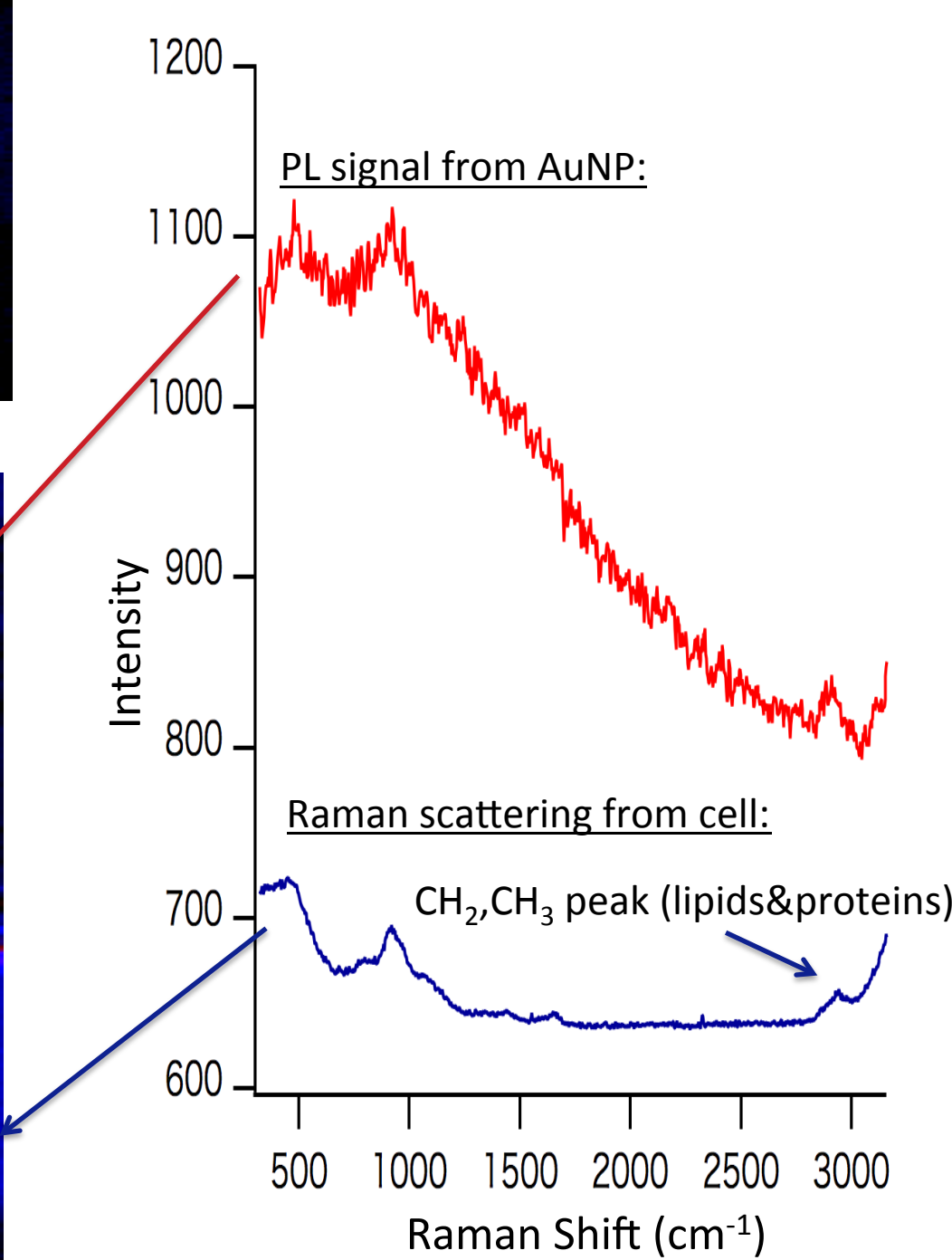


Fig. 2: (above) Example spectra for strong PL signal from AuNP and Raman scattering from HeLa cell. Excitation wavelength 532 nm.

Optimization of Electroporation Parameters by PL Imaging

Poring Pulse	75V, 10 ms pulse x 1 0% pulse decay, +	115V, 10 ms pulse x 2 0% pulse decay, +	115V, 7.5 ms pulse x 2 10% pulse decay, +
Transfer Pulse	75V, 1 ms pulse x 1 0% pulse decay, +	75V, 1 ms pulse x 1 0% pulse decay, +	20V, 50 ms pulse x 5 40% pulse decay, +/-
Electroporation	(a)	(b)	(c)
Without Electroporation (control)	(d)	(e)	(f)

Fig. 3: PL images of NPs in HeLa cells with electroporation, (a)-(c), and without electroporation, (d)-(f), after 30 minutes. Green color shows PL hot spots from AuNPs, and red color shows outline of cells. Excitation wavelength 532 nm.

SERS Imaging of Electroporation-introduced AuNPs in HeLa Cells

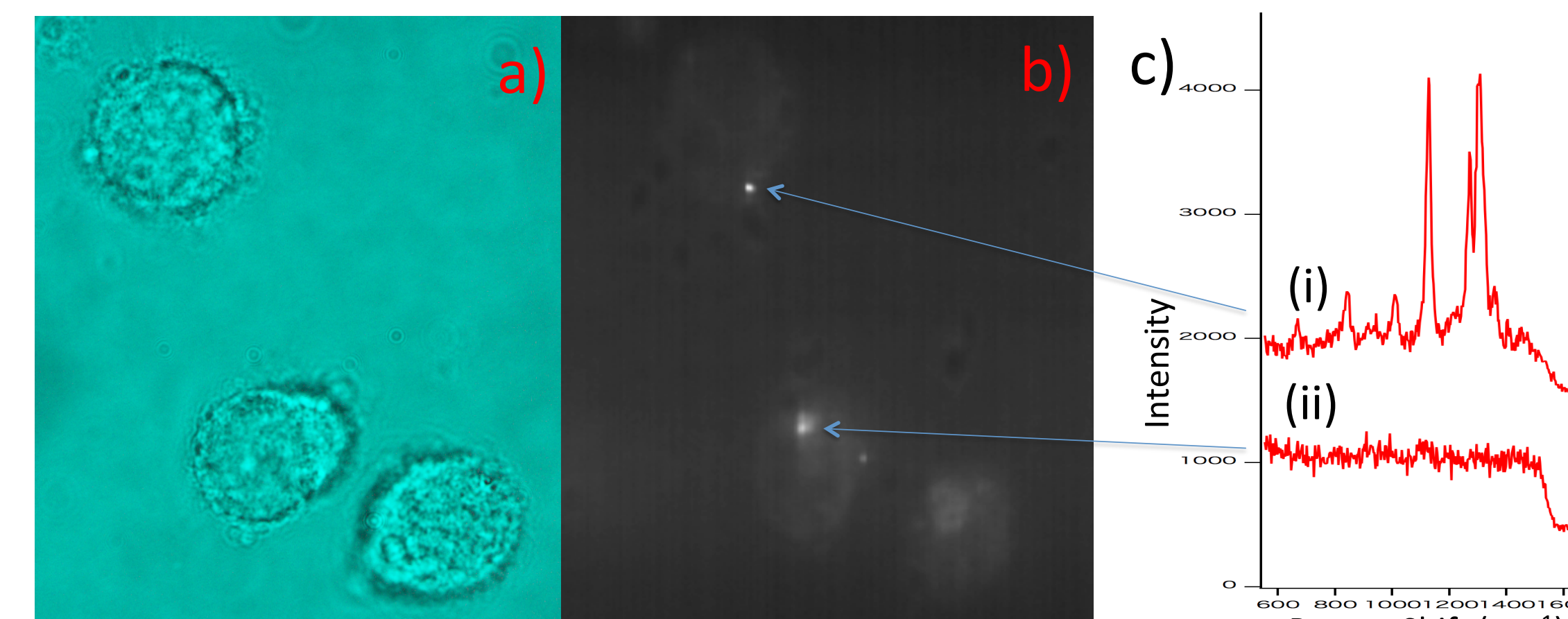


Fig. 4 (above) : (a) Brightfield image and (b) SERS image of HeLa cells after electroporation. Graph (c) shows SERS spectrum (i) and PL signal (ii). Excitation wavelength 590 nm.

Comparison of SERS Spectra

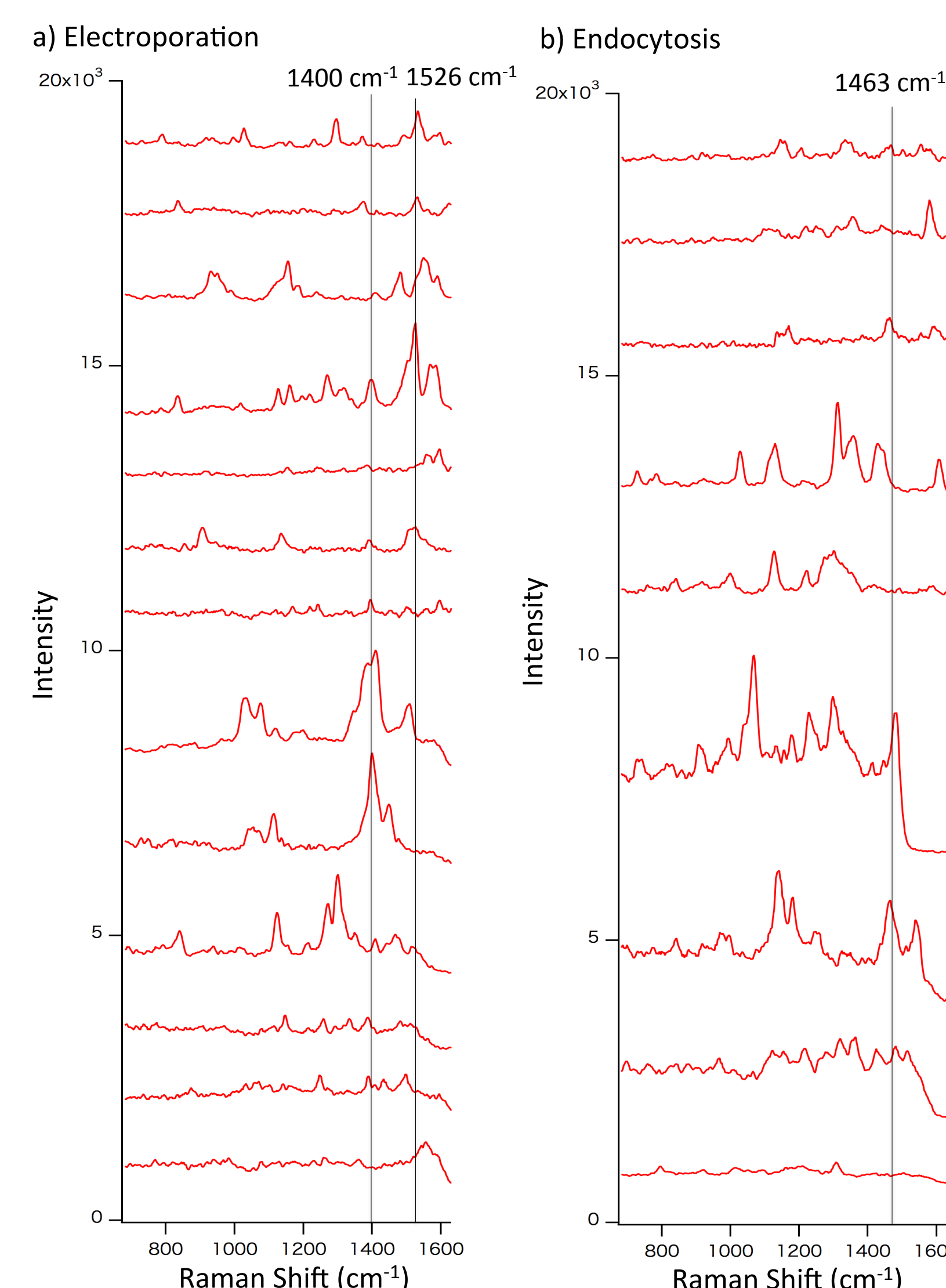


Fig. 5: SERS spectra collected from cells (a) 30-60 minutes after electroporation and (b) after three hours of incubation for endocytosis. Excitation wavelength 590 nm. Characteristic peak wavenumbers (see fig. 6) marked.

Comparison of Average SERS Spectra

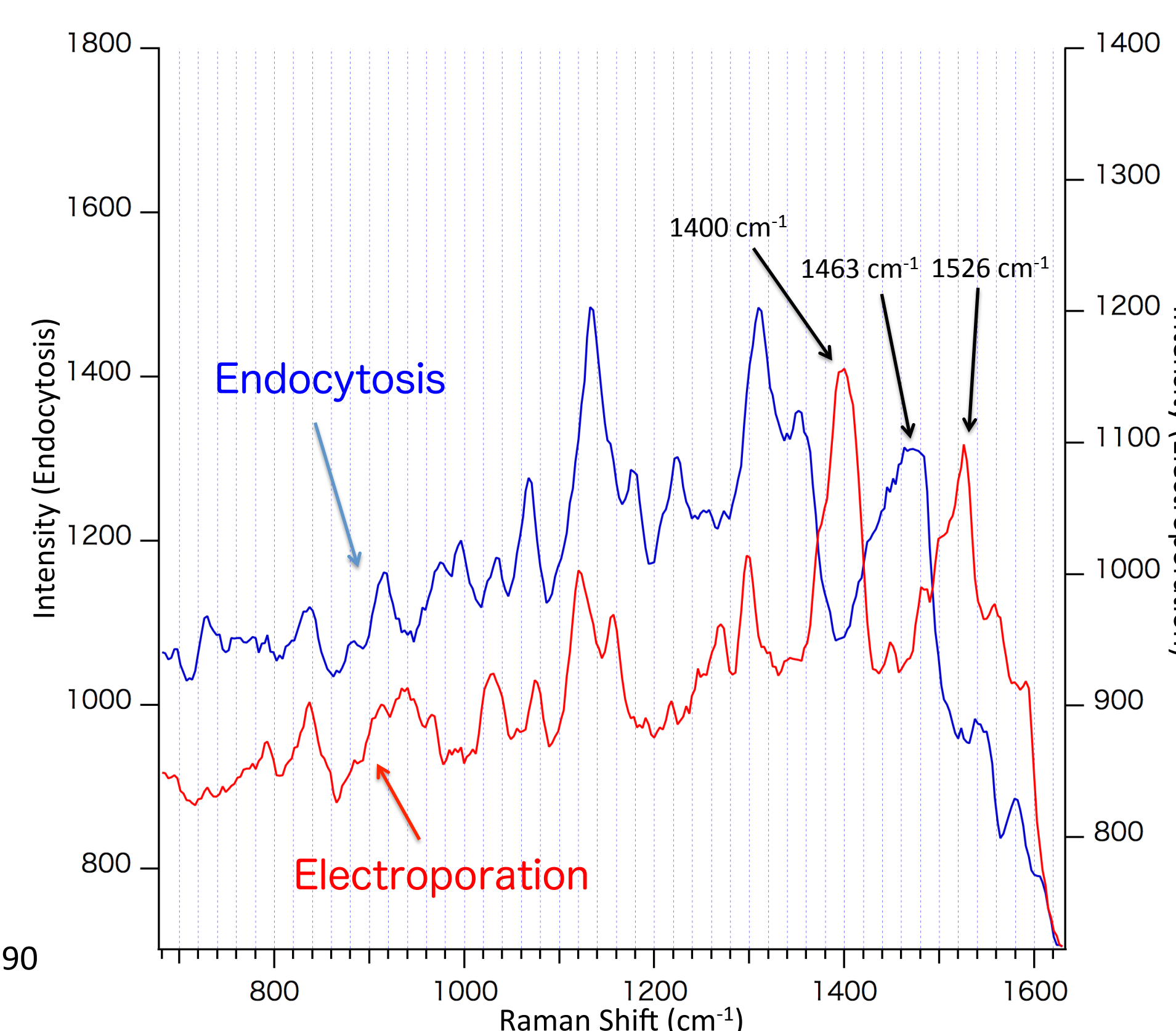


Fig. 6 (left) : Average spectra calculated from SERS data (fig. 5). Several peaks which appear characteristic of either electroporation or endocytosis are marked. Tentative assignments for these bonds are below.

Tentative peak assignments:

Wavenumber (cm⁻¹)	Tentative Assignment
1400	COO: protein
1463	CH ₂ CH ₃ : lipid
1526	A, C, G: base

DISCUSSION

- Established electroporation parameters for introduction of 50 nm AuNPs into suspended HeLa cells
- PL Imaging with 532 nm excitation laser provided proof of particle entry despite high lipid vesicle content of HeLa cells
- Average of SERS data from electroporation and endocytosis samples showed several characteristic peaks which distinguish the two methods
- Tentative peak assignments suggest electroporation-introduced particles are exposed more frequently to proteins and A, C, G bases of nucleic acids, consistent with particles in cytoplasm, while endocytosed particles are more frequently exposed to lipids, consistent with vesicle environment

NEXT STEPS

- Introduction of different sizes, shapes of AuNPs by electroporation
- Study of cellular effects of electroporation including viability
- Time-lapse PL/SERS imaging of electroporation-introduced NPs in cells

CITATIONS

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- Kneipp, et al., NanoLetters. 6(10), (2006).
- Fujita, et. al., Journal of Biomedical Optics 14(2), (March/April 2009)

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