Simultaneous Detection of SERS and Fluorescence Signals Using a Single Excitation Laser Source for Microbead-Based Analysis

Sa Ram Lee[1], Chang Su Jeon[2], In Seong Hwang[2], Taek Dong Chung[2], and Hee Chan Kim[3,4*]

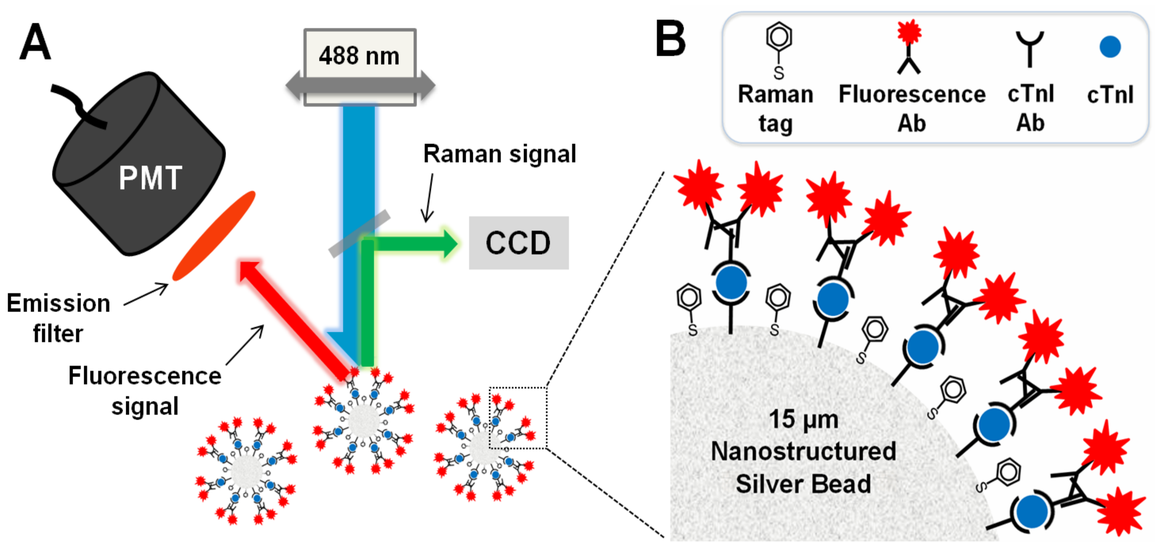
[1] Interdisciplinary Program, Bioengineering Major, Graduate School, Seoul National University, Seoul 110-744, Korea
[2] Departments of Chemistry, Seoul National University, Seoul 151-742, Korea
[3] Departments of Biomedical Engineering, College of Medicine, Seoul National University, Seoul 110-744, Korea
[4] Institute of Medical & Biological Engineering, Medical Research Center, Seoul National University, Seoul 110-744, Korea

INTRODUCTION

The rapid advance in biochemical studies, clinical diagnostics, and environmental monitoring keeps stimulating the demand in powerful analytical methodology for collecting huge information from smaller sample volume, in shorter reaction time, and at lower cost. As such, multiplex assay using bead-based suspension array has gathered much attention in that it provides faster binding kinetics, lower costs, higher reproducibility, and superior detection sensitivity.

Herein, we report a simple barcoding strategy using individually functionalized microbeads that can be applied to array-based multiplex analysis. Our strategy exploits a simultaneous detection of SERS and fluorescence signals using a single excitation source.

METHODOLOGY



* Schematic illustrations of experimental setup (figures not drawn to scale).

A. Simultaneous Detection of SERS and fluorescence

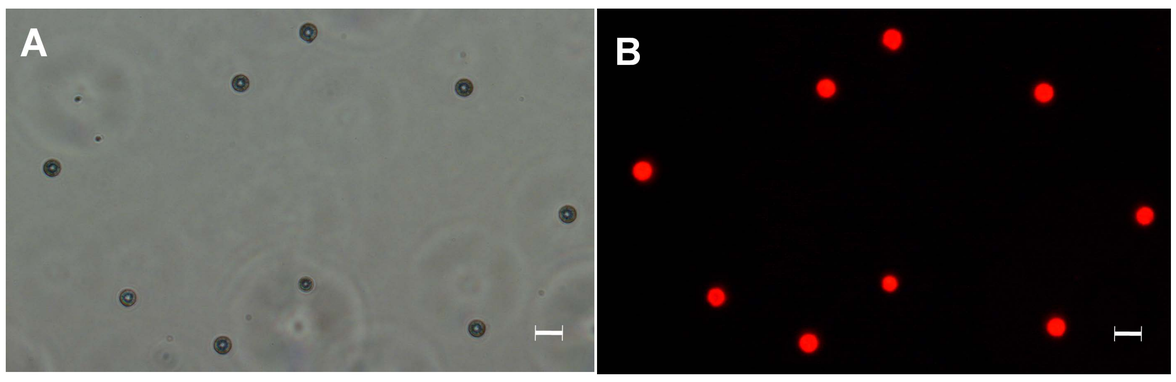
- Excitation source
  Argon laser (488 nm) with a beam diameter of 2 μm
- Fluorescence
  PMT with emission filter(624nm)
- SERS
  Cooled charge coupled device (CCD)

For simultaneous detection of SERS and fluorescence signals from multiple individual silver microbeads, the beads were spread out on a glass slide and scanned with a laser.

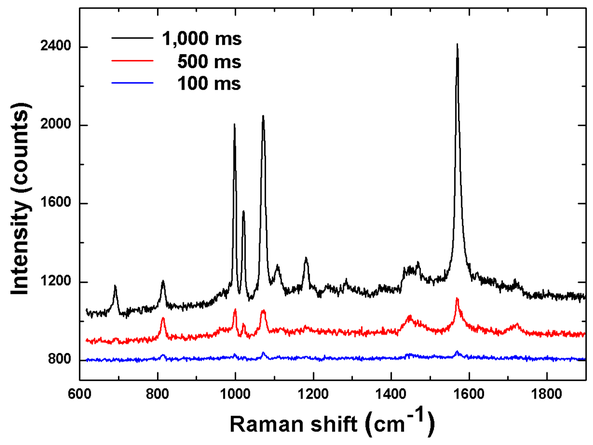
B. Functionalized microbeads preparation

- 15 μm PMMA bead
- Electroless plating method with silver nanoparticles (AgNPs, 2–3 nm)
- Raman tag
  1 mM benzenethiol (BT)
- Antibodies and antigen
  5 ng of rabbit anti-cTnI antibody (Abcam)
  cardiac troponin I (cTnI)
  10 ng of mouse anti-cTnI antibody (GeneTex).
  25 ng of AlexaFluor 610-PE goat anti-mouse IgG (Invitrogen)

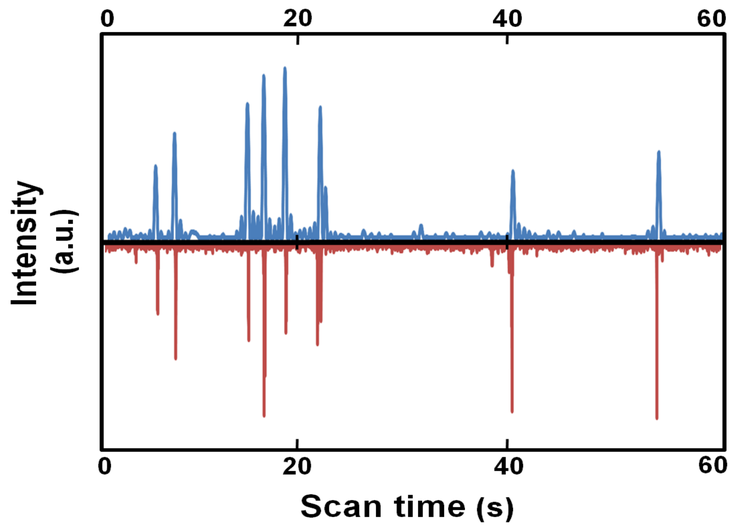
RESULTS



* Representative microscopy image of functionalized silver microbeads. It shows the binding of cTnI, a specific biomarker for myocardial-ischemia, with antibody on the bead surface. (a) A light image. (b) A fluorescence image of sandwich immunoassay using 0.4 ng/mL cTnI cardiac marker proteins. Scale bars are 20 μm.



* SERS spectra with various laser exposure time ranging from 100 ms to 1 s. The Raman tag (BT) chemisorbed on the surface of functionalized silver microbead can be clearly identified from its characteristic peak at 1,020 cm^{-1} with an exposure time of 100 ms.



* SERS (upper) and fluorescence (lower) signals from a single silver microbead exactly coincide when simultaneously detected by laser scanning on a glass slide. The peak at 1,020 cm^{-1} was monitored as a representative SERS peak and fluorescence signals were filtered through 624 ± 20 nm band pass filter.

DISCUSSION

The in situ decoding and detection of both SERS and fluorescence are one of the promising strategies that deserve intensive efforts to practical applications for biological and chemical analyses. Here, we successfully demonstrated the facile fabrication of functional silver microbead for simultaneous detection of SERS and fluorescence using a single excitation using 488 nm laser source. The SERS signals from a given Raman tag could be detected without interaction with fluorescence emission signals. Thus, our system shows the possibility of a simple barcoding strategy and multi-functional microbeads for high-throughput multiplex analysis.

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