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Optimization procedures for development of SERS-based lateral flow assay for high sensitive detection of Troponin I

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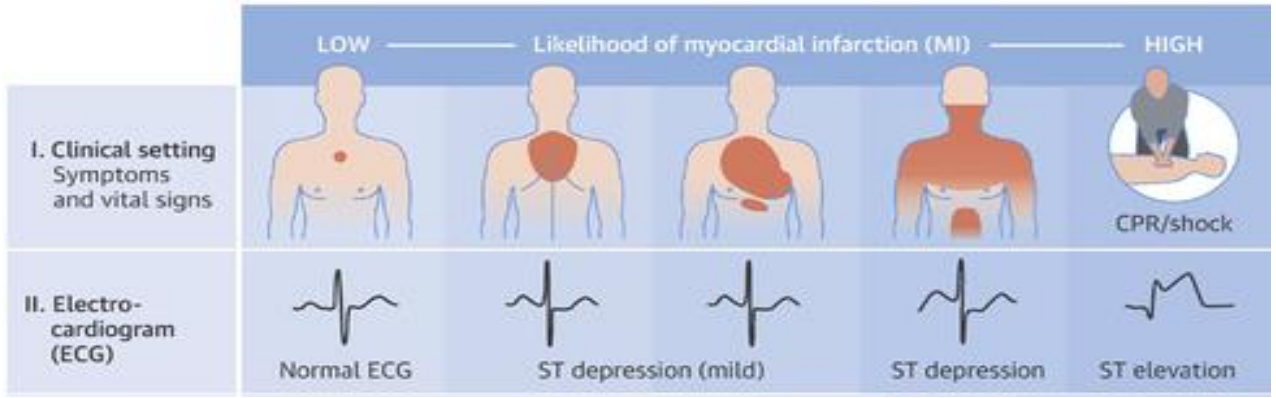
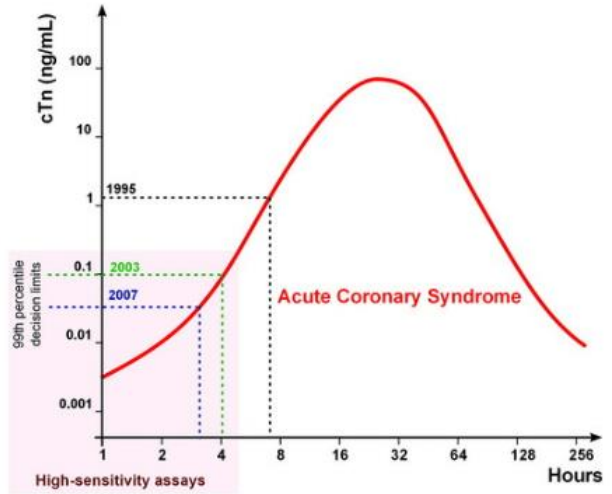
Content

Introduction



J Am Coll Cardiol 2017; 70:996-1012

Figure 1. Patient assessment with suspected acute coronary syndrome (ACS)



Circulation 2011; 124,2350-2354

Figure 2. Evolution of the cardiac troponin (cTn) assays and their diagnostic cutoffs.

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Commercial cTnI LFA test

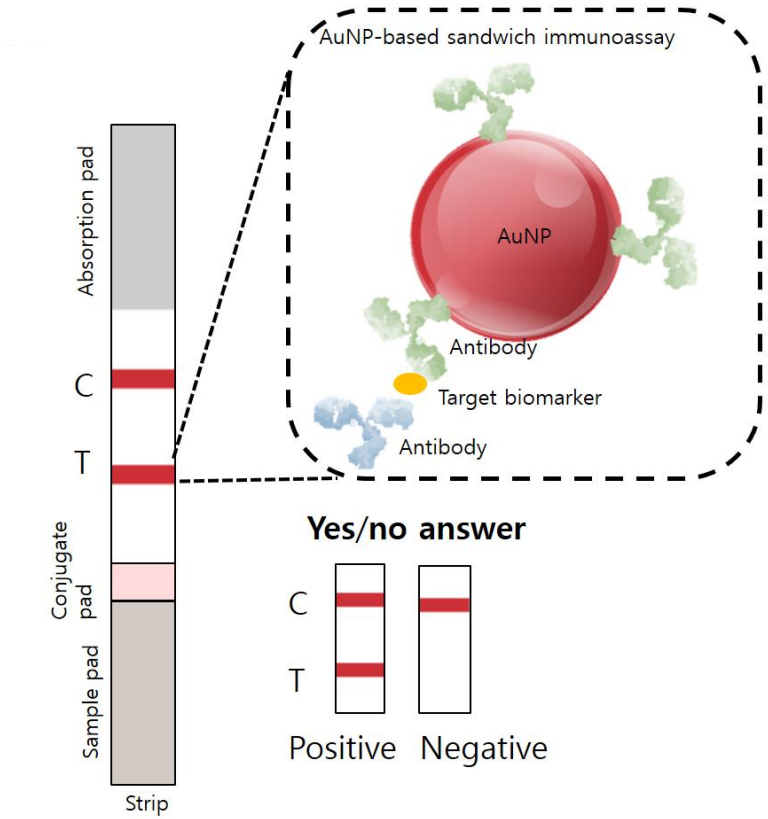

Figure 3. Commercial cTnI LFA test provides only 'yes/no answer'

Analytical performance of cTnI immunosensor

- Chemiluminescence
- Fluorescent
- Electrochemistry
- Surface-enhanced Raman spectroscopy

- Short detection time
- Simple procedures for quantitative assay after cTnI LFA result comes out
- Ultra sensitivity

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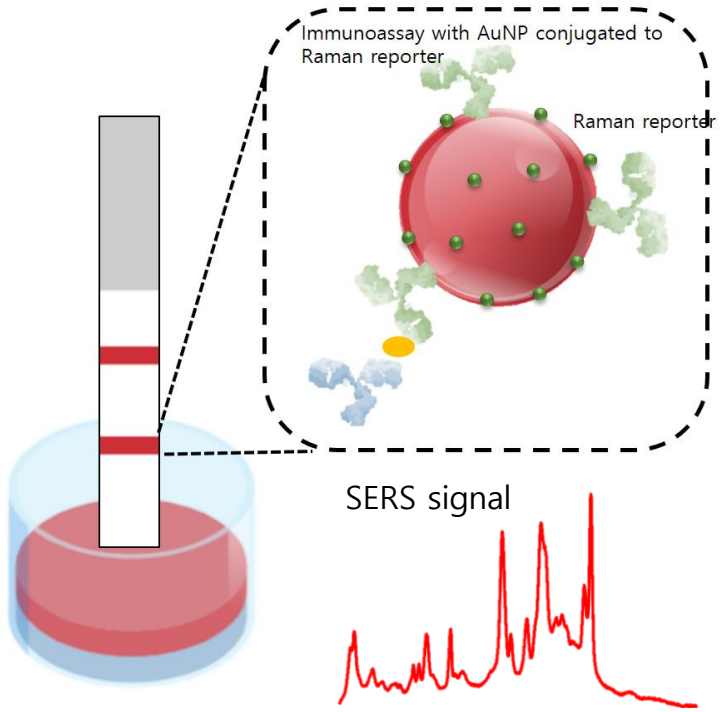
Design of this experiment



Optimizing gold nanoparticle sizes for SERS quantitative assay on LFA

Investigating LFA components and fluid flow time with SERS performance

Evaluating different laser wavelengths and laser power for SERS-based LFA

Figure 4. Working principle of ultra sensitive SERS-based LFA platform obtained the intensity from raman reporter conjugated on Au nanoparticles

Method

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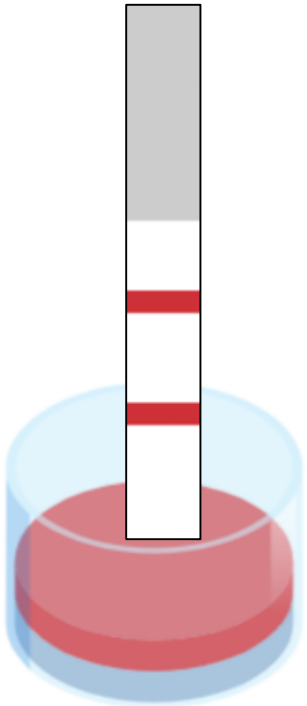

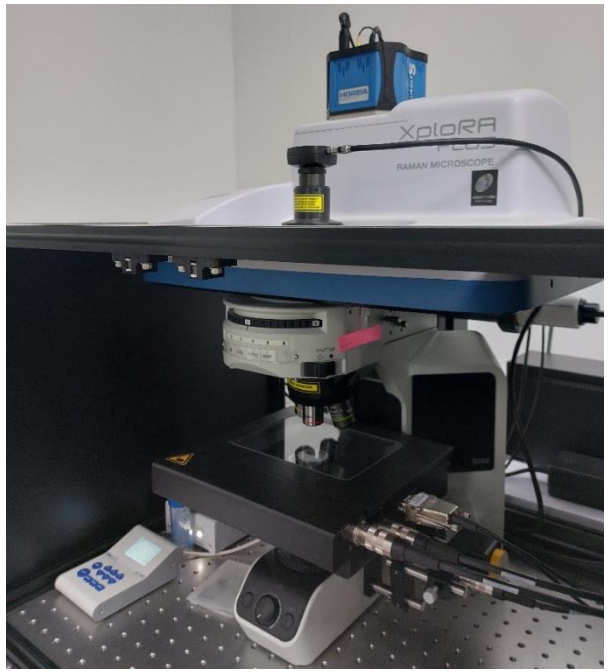

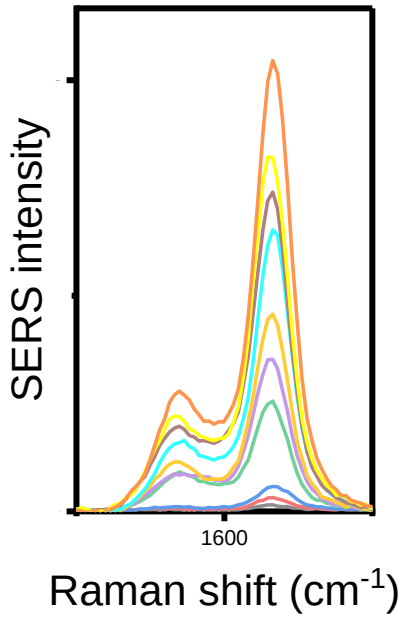

Dipping LFA strip in sample results 'yes/no answer'

1 min analysis for SERS-based assay with 105 pixels on each test strip

Quantitative assay

Figure 5. Process of SERS-based LFA. Traditional way dipping LFA strip in a sample results in 'yes/no answer' and test region of LFA used for quantification with SERS-based assay. Quantitative assay takes only 1 min.

Result and Discussion

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Optimization of gold nanoparticle size on SERS-based LFA for detection of cTnI

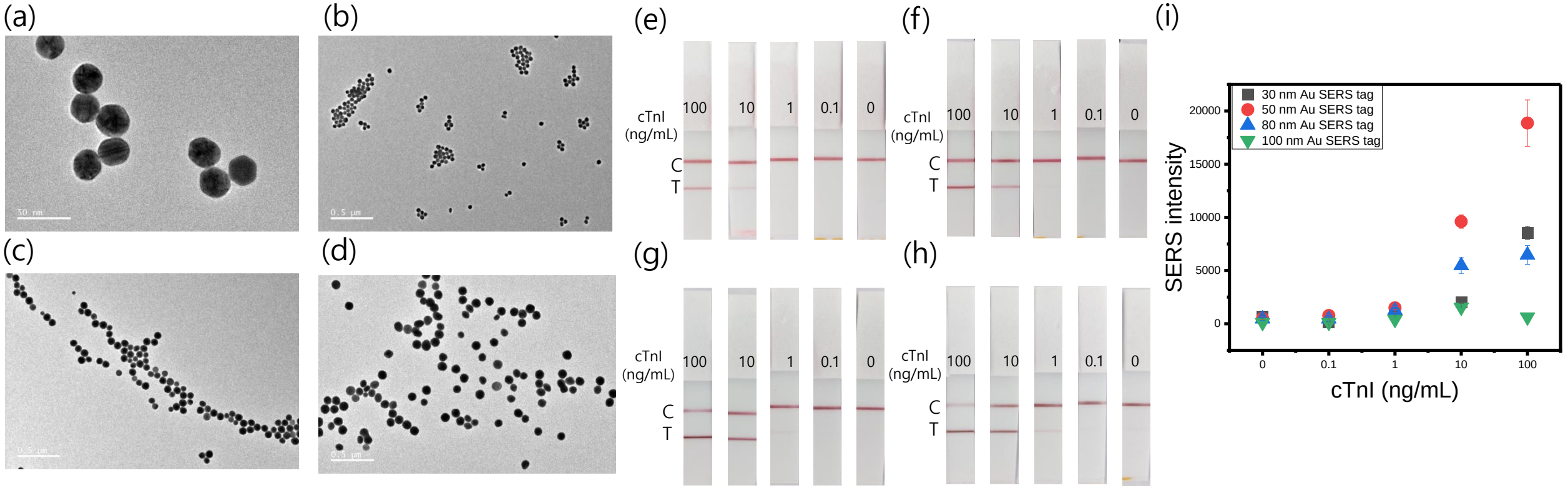

Figure 6. TEM images of Au nanoparticles with sizes of 30 (a), 50 (b), 80 (c), and 100 (d) nm. LFA test results achieved with different Au sizes of 30 (e), 50 (f), 80 (g), and 100 (h) nm as SERS tags. (i) Quantification of the cTnI concentration in SERS-based LFA.

Optimization of the running buffer formulation and sample loading time for SERS-based LFA

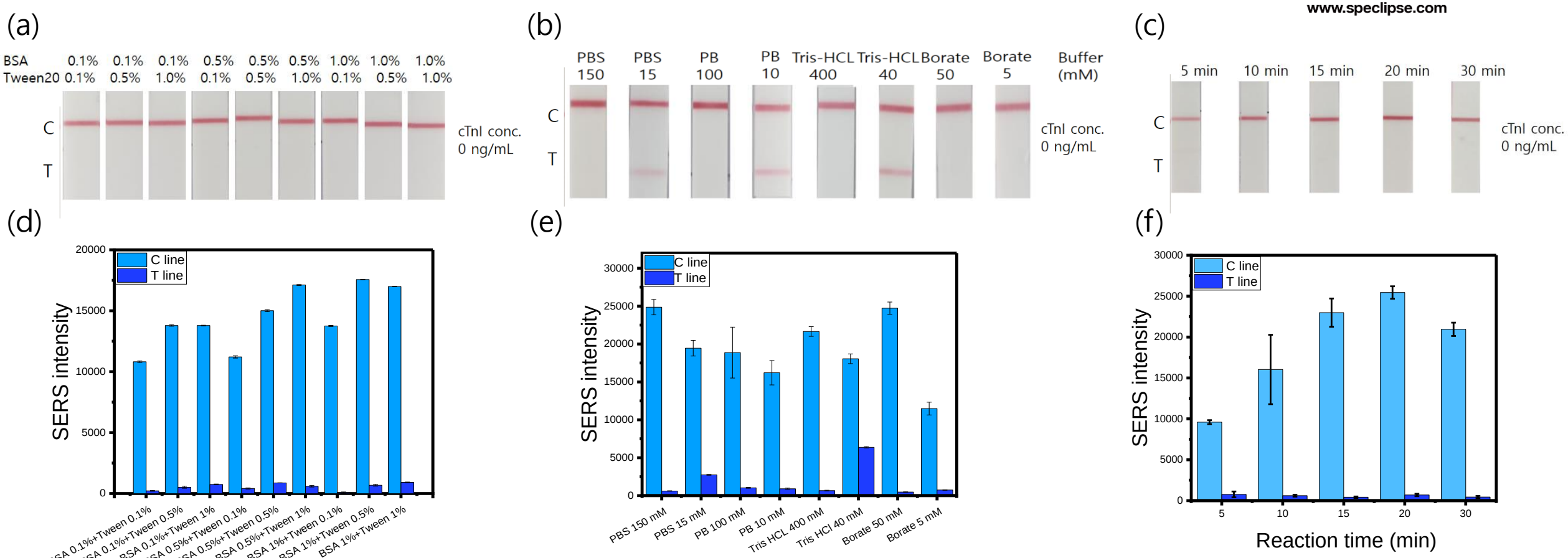

Figure 7. LFA result images for (a) protein and surfactant test in running buffer, (b) buffer type and concentration test, and running time test. SERS performance on test region and control region – (d) protein and surfactant impacts in running buffer, (e) buffer type and concentration test, and (f) running time test

Optimization of laser wavelengths and laser power for SERS-based LFA for the analysis of Troponin I

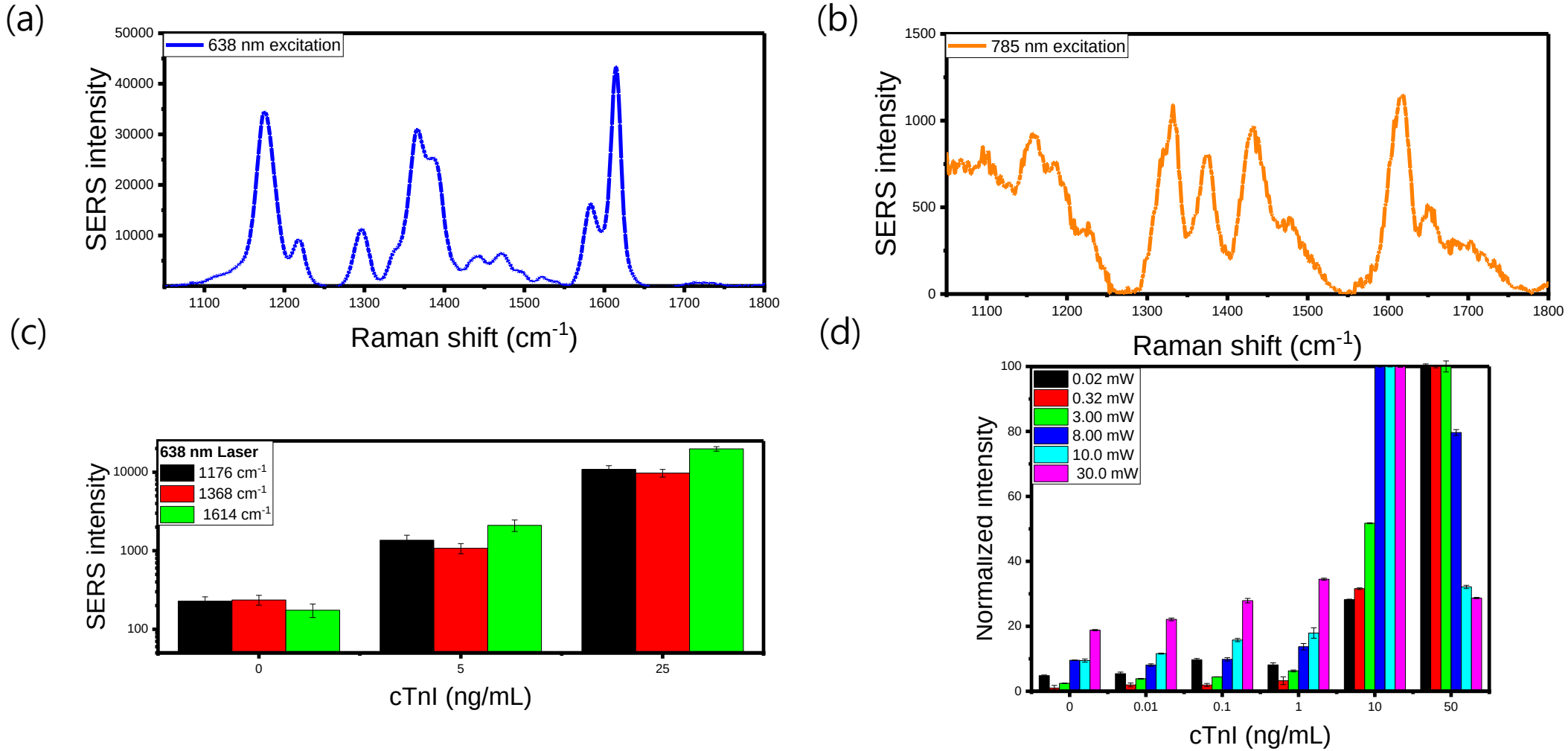

Figure 8. Laser wavelength effects: (a) 638 nm and (b) 785 nm excitation with 3 mW laser set on the cTnI LFA test region; (c) comparison of SERS intensities at 1175 cm^{-1}, 1368 cm^{-1}, and 1614 cm^{-1}; and (d) laser power varied from 0.02 mW to 30 mW for SERS-based LFA.

Performance evaluation of SERS-based LFA for the detection of cTnI

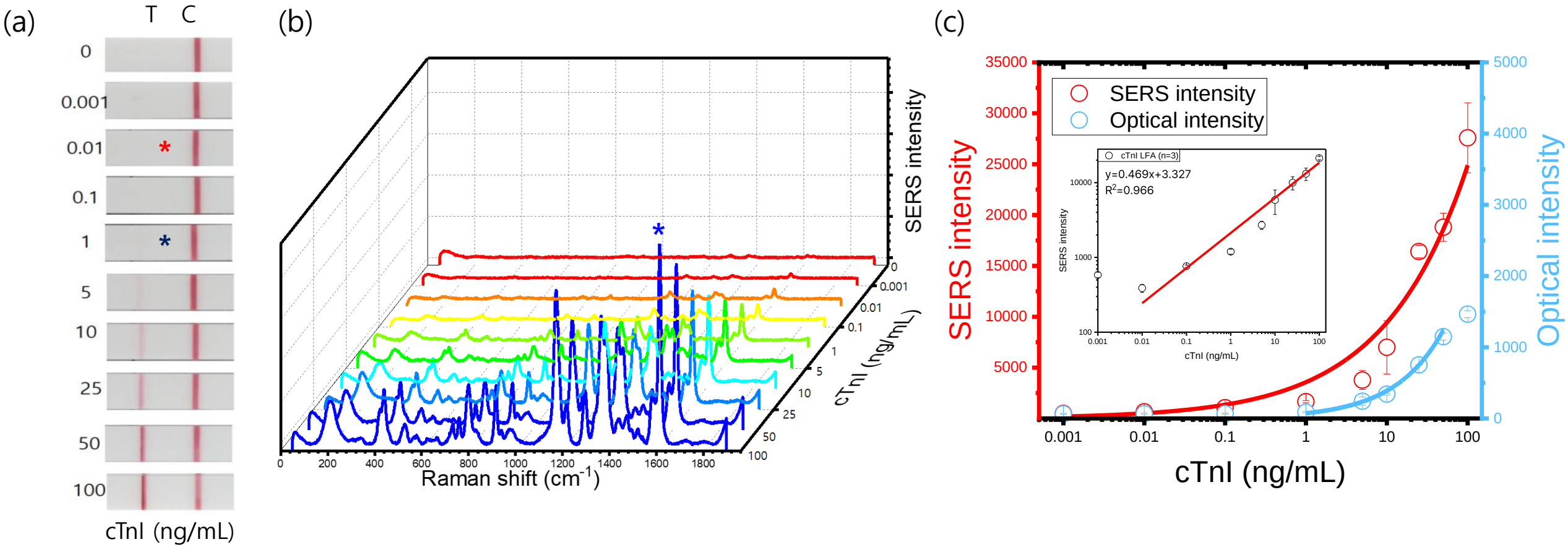

Figure 9. (a) cTnI LFA test result after all parameters were optimized. The red and dark blue asterisks indicate the lowest concentration of samples distinguishable by SERS intensity and optical intensity, respectively. (b) Average SERS spectra. The blue asterisk indicates the peak at 1614 cm^{-1} for SERS intensity. (c) Calibration curves of optical intensity and SERS intensity for the test line with 50 nm Au SERS tag loaded on cTnI LFA.

Selectivity test

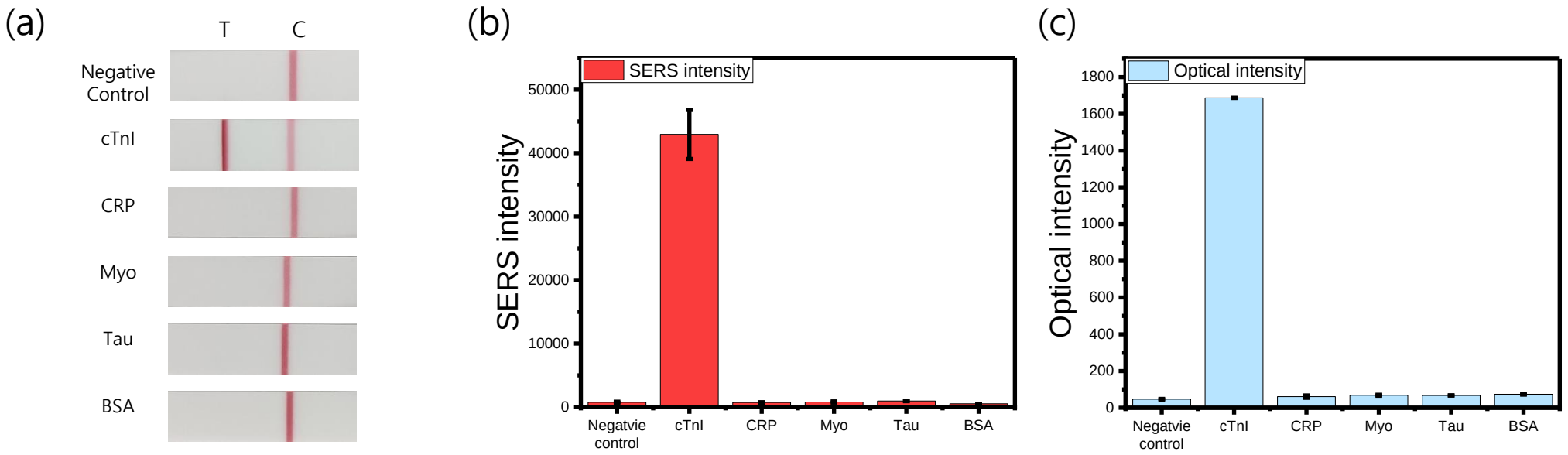

Figure 10. Selectivity test results. (a) LFA test results for 10 ng/mL solutions of different target proteins. (b) SERS intensity and (c) optical intensity for the selectivity test.

Evaluation of SERS-based LFA for the detection of cTnI in human serum

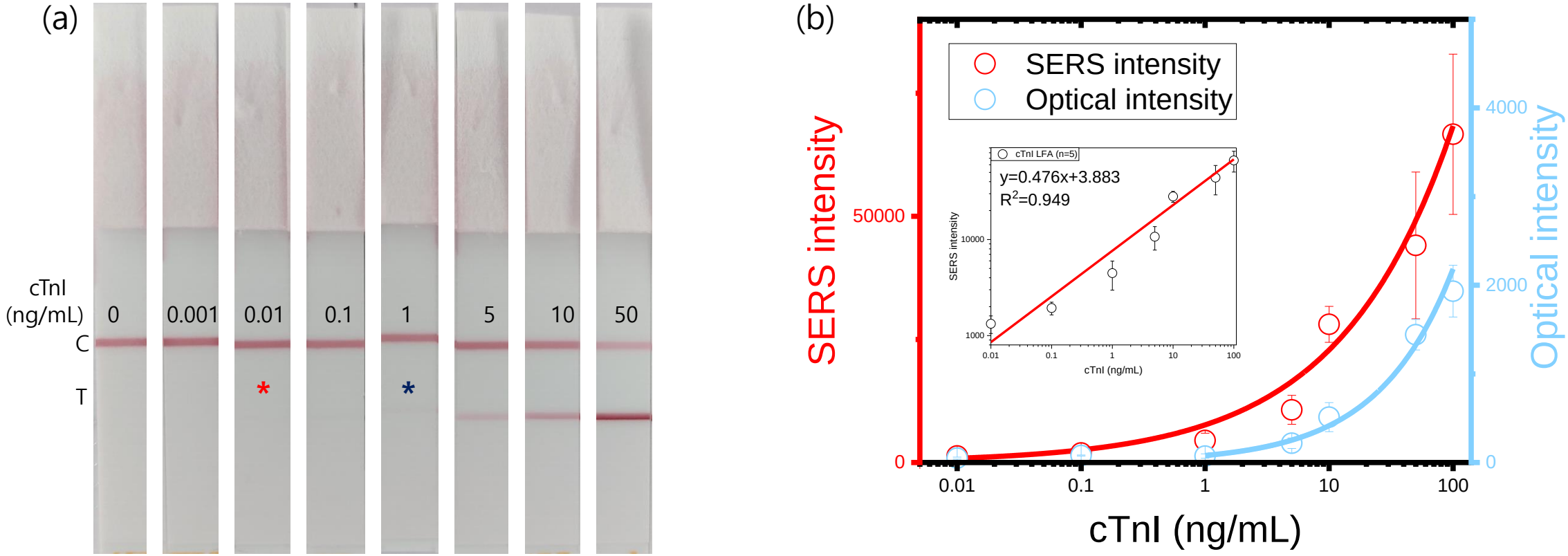

Figure 11. (a) Visual images obtained after loading the sample onto the LFA. The red asterisk indicates the lowest concentrations of the sample that are distinguishable by SERS-based LFA, and the dark blue asterisk indicates the lowest concentration of the sample that is distinguishable by optical intensity, while 5ng/mL cTnI was distinguishable with the naked eye. (b) SERS intensity and optical intensity plot obtained from the cTnI LFA test (Inset: Calibration curve for SERS based cTnI LFA)

Conclusions

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- 50 nm Au SERS tag had adequate properties of sensitivity and stability on SERS-based LFA for detection of cTnI, compared with 30 nm Au, 80 nm Au and 100 nm Au SERS tags.
- Three critical points on LFA components as running buffer solution type, surfactant and protein contents and sample loading time were optimized as PBS 150 mM, 1% Tween 20 and BSA, and 15 min reaction time.
- 638 nm laser wavelength rather than 785 nm laser were adequate for quantitation and selected peak data at 1614 cm^{-1} showed upmost strong intensity in MGITC raman reporter on 50 nm Au. With the condition of 0.25s acquisition time of laser, 3 mW laser power were selected for SERS performance.
- SERE-based LFA compared with optical intensity can detect approximately100 times lower concentration of cTnI after optimized parameters, revealing the goodness of SERS performance by covering the cut-off level for ultra sensitive target detection.

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