Supporting Information

"Reporter selection for Nanotags in Multiplexed Surface Enhanced Raman Spectroscopy Assays"

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SERS-encoded GNS characterization

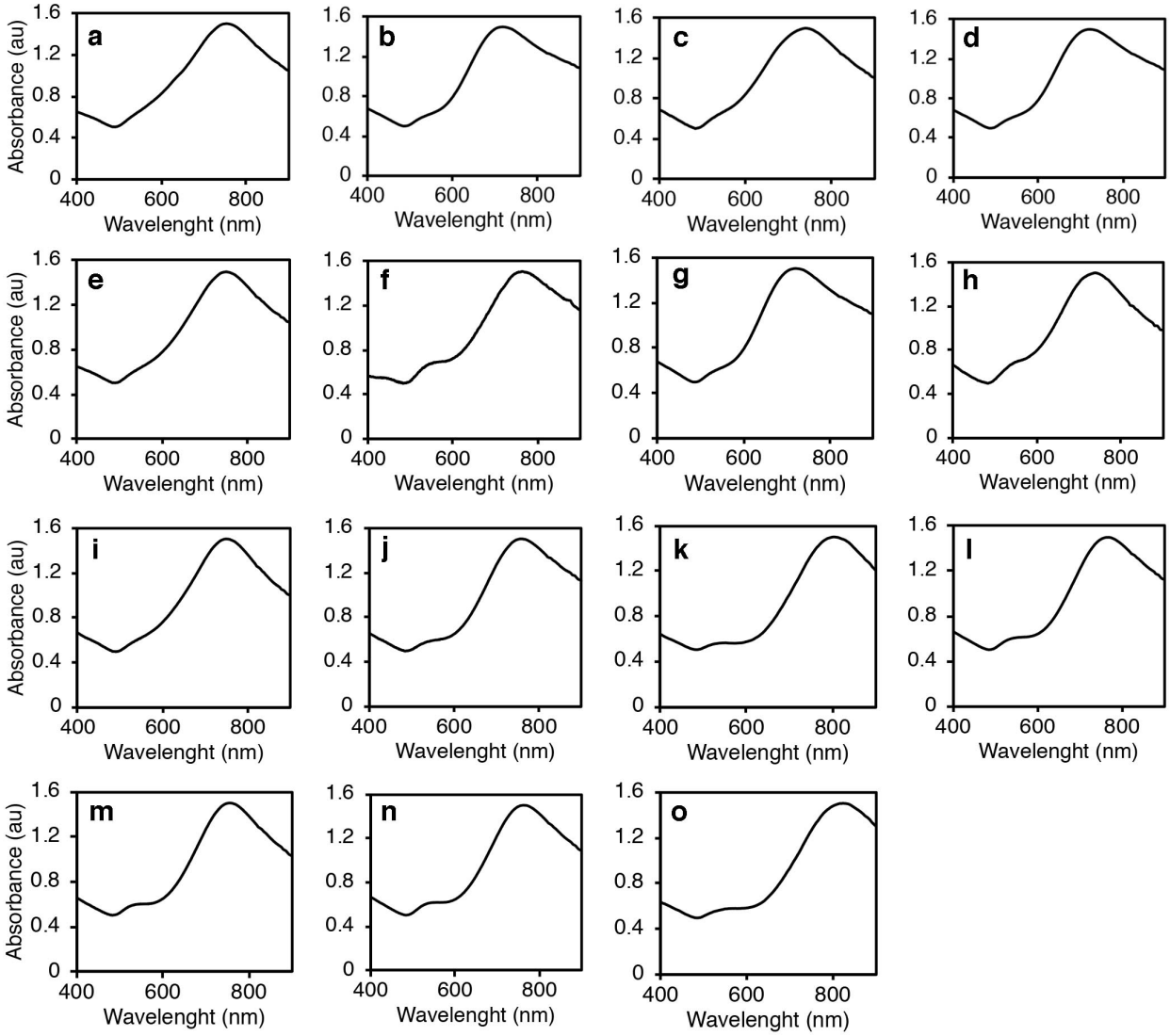


Figure S1: Optical characterization of SERS-encoded GNS. Vis-NIR spectra of GNS-Rep-PEG for the 15 selected Raman reporters: GNS-BCB-PEG (a), GNS-CV-PEG (b), GNS-MB-PEG (c), GNS-MG-PEG (d), GNS-MEG-PEG (e), GNS-NR-PEG (f), GNS-RB-PEG (g), GNS-R6G-PEG (h), GNS-VB-PEG (i), GNS-ATP-PEG (j), GNS-BPE-PEG (k), GNS-MBA-PEG (l), GNS-DCT-PEG (m), GNS-PCTP-PEG (n) and GNS-DTNB-PEG (o).

Aggregation Index of GNS conjugates

The aggregation index (AI) of GNS was calculated for plain nanostars (GNS) and after adding reporter (GNS-Rep), antibody (GNS-Rep-Ab) and PEG backfill (GNS-Rep-Ab-PEG), as a quantitative measure of their colloidal stability. The AI is a measure of the longitudinal surface plasmon resonance (LSPR) peak broadening derived from the total area under the absorption spectrum of the LSPR from 600 to 1000 nm, divided by LSPR intensity.[1] A higher degree of aggregation corresponds to a higher AI value.

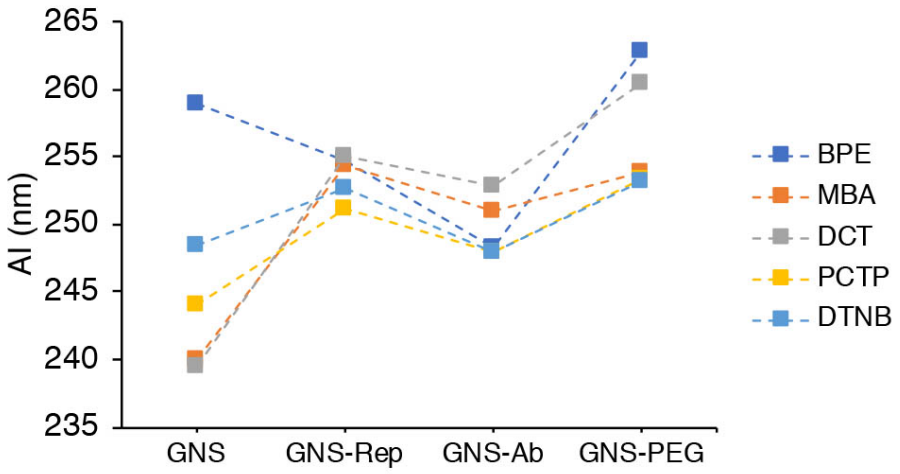


Figure S2: Aggregation index of GNS, GNS-Rep, GNS-Rep-Ab and GNS-Rep-Ab-PEG for the 5 seleted nanotags from Figure 3.

Antibody coverage determination

To determine the number of Antibody molecules/GNS, the amount of total antibody present in each sample was measured by the Bicinchoninic acid assay (BCA assay). The standard curve was performed with initial concentrations of 50 μ g/ml of BSA with subsequent 2-fold dilutions to obtain 7 points.

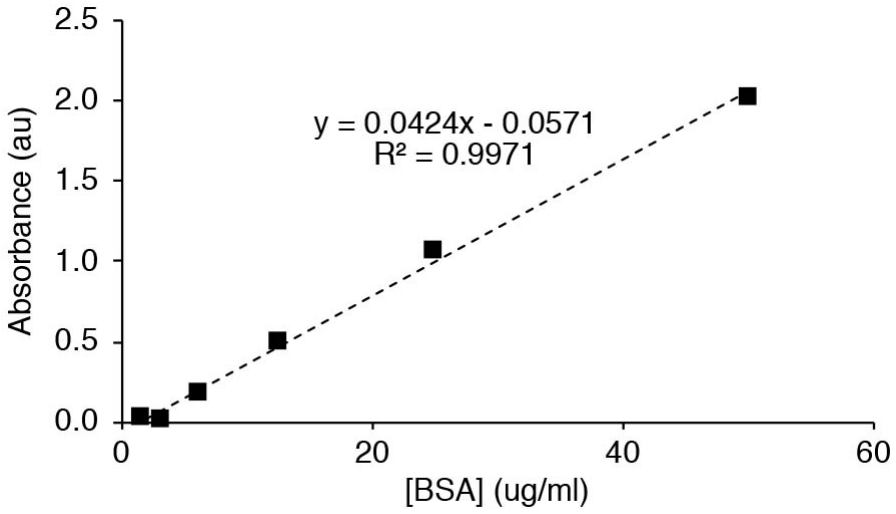


Figure S3: BSA Calibration curve for the Bicinchoninic acid assay (BCA assay) at 562 nm.

Dipstick immunoassay

To make sure the positive tests are not a result of the non-specific interactions between the nanotags and the printed antibody, negative controls were run individually for each nanotag. In the negative controls, IgG was substituted by the same volume of PBS 1X. Figure S1a shows negative tests for the five controls (no spot on the test line). SERS spectra of the test line was measured for each test (Figure SFigure S1b). All the test showed the spectrum profile of nitrocellulose, which individually shown below (NC spectrum). When the test is positive and nanotags are present on the test, their signal intensity were high enough to obscure high that nitrocellulose peaks cannot be seen.

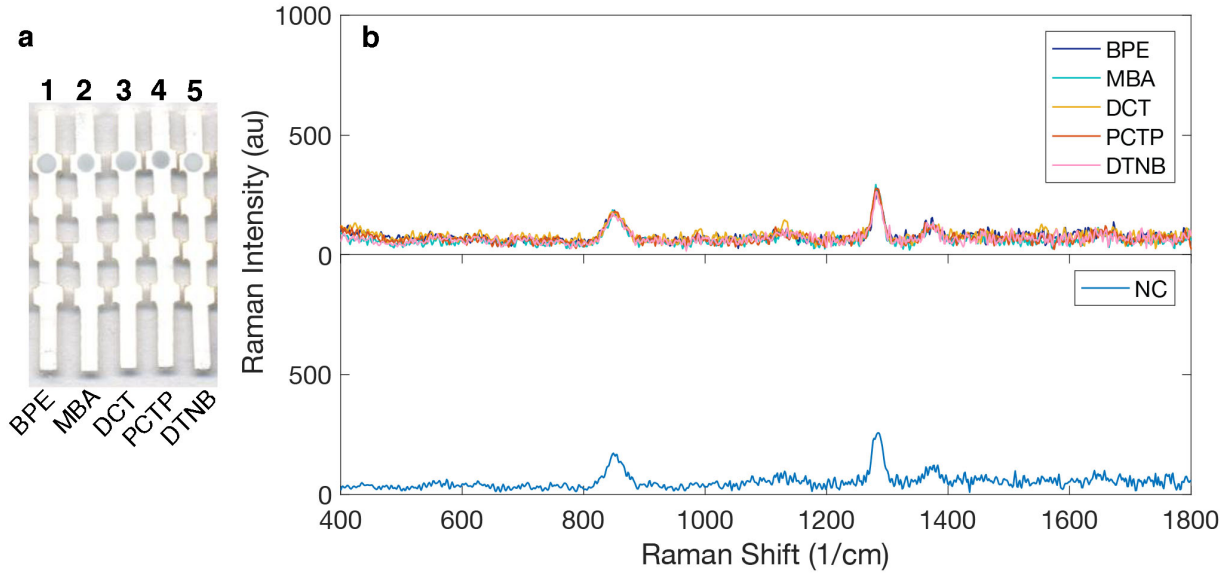


Figure S4: Flow immunoassay negative control. a) Negative controls of individual test with the 5 nanotags (strips 1-5). b) SERS spectra of the test line of the 5 strips and nitrocellulose spectrum (NC) from Nitrocellulose sample as supplied.

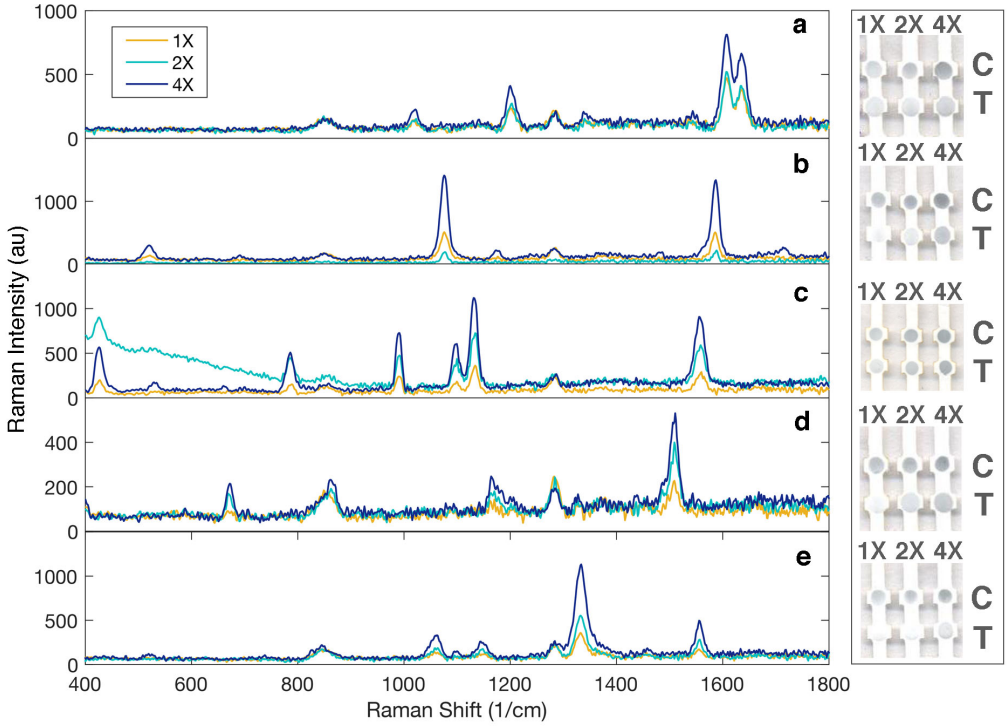


Figure S5: SERS spectra of individual assays varying nanotags concentration. SERS spectra of the test line from the 5 nanotags with increasing concentration of nanotag (1X, 2X and 4X). a) BPE, b) MBA, c) DCT, d) PCTP and e) DTNB nanotags.

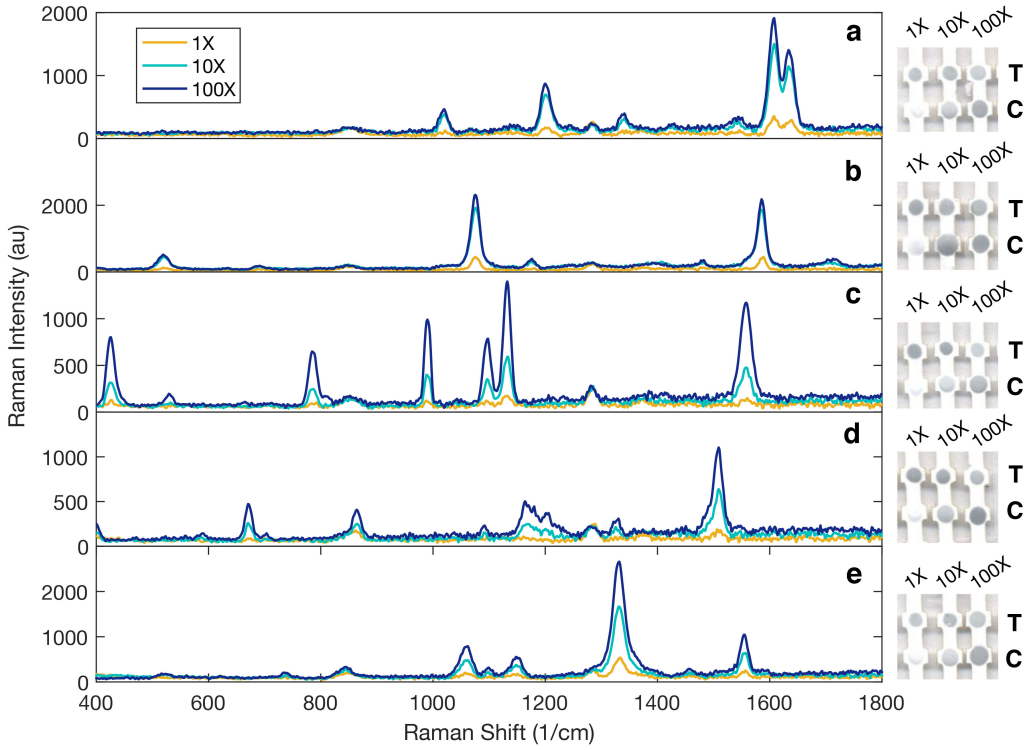


Figure S6: SERS of individual assays varying antigen concentration. SERS spectra of the test line from the 5 nanotags with increasing concentration of IgG (1X, 10X and 100X). a) BPE, b) MBA, c) DCT, d) PCTP and e) DTNB nanotags.

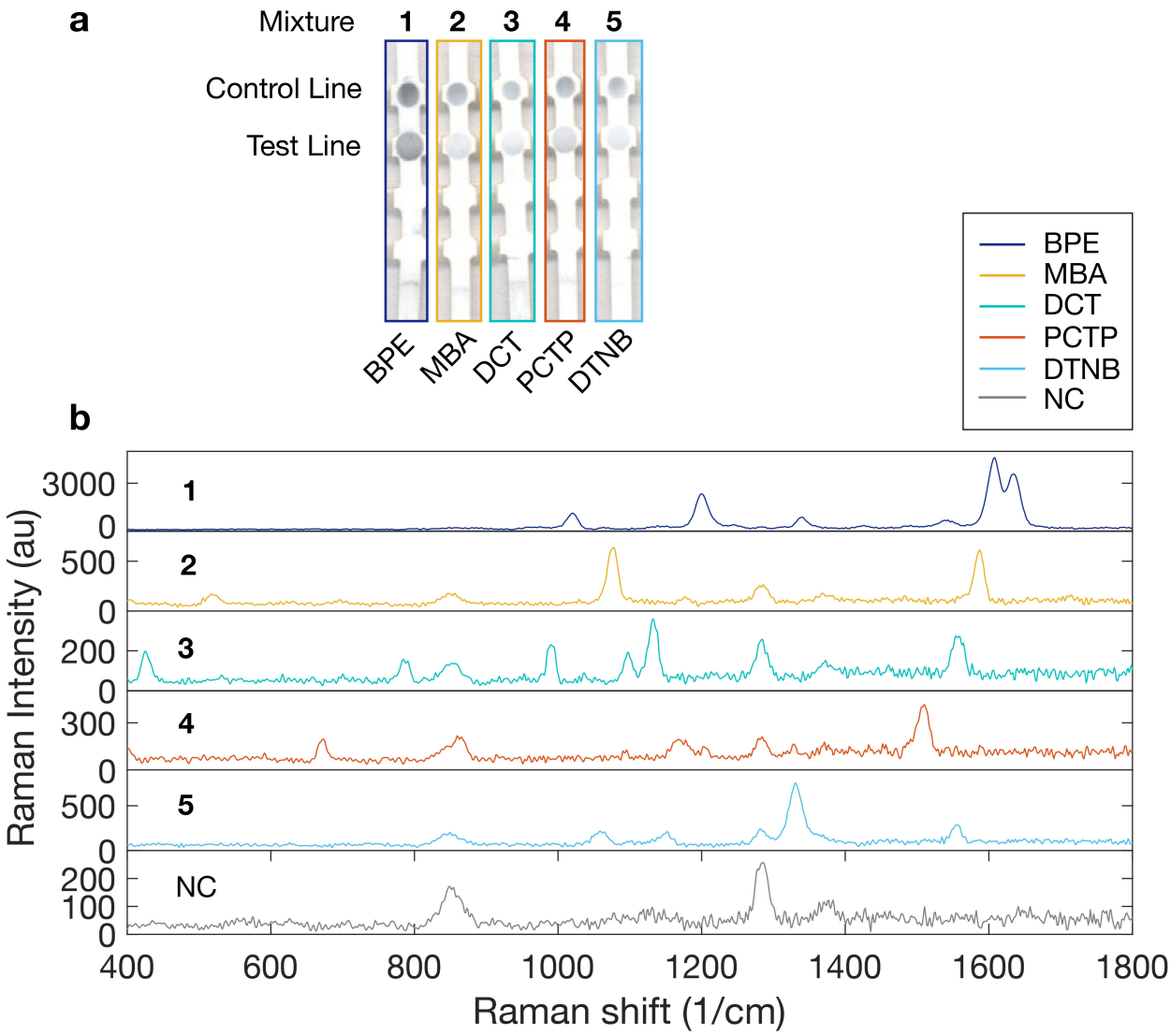


Figure S7: Sandwich immunoassay with single nanotags (testing set). a) Resulting strips from running individual nanotags (strips 1-5). b) SERS spectra of the 5 nanotags and nitrocellulose (NC).

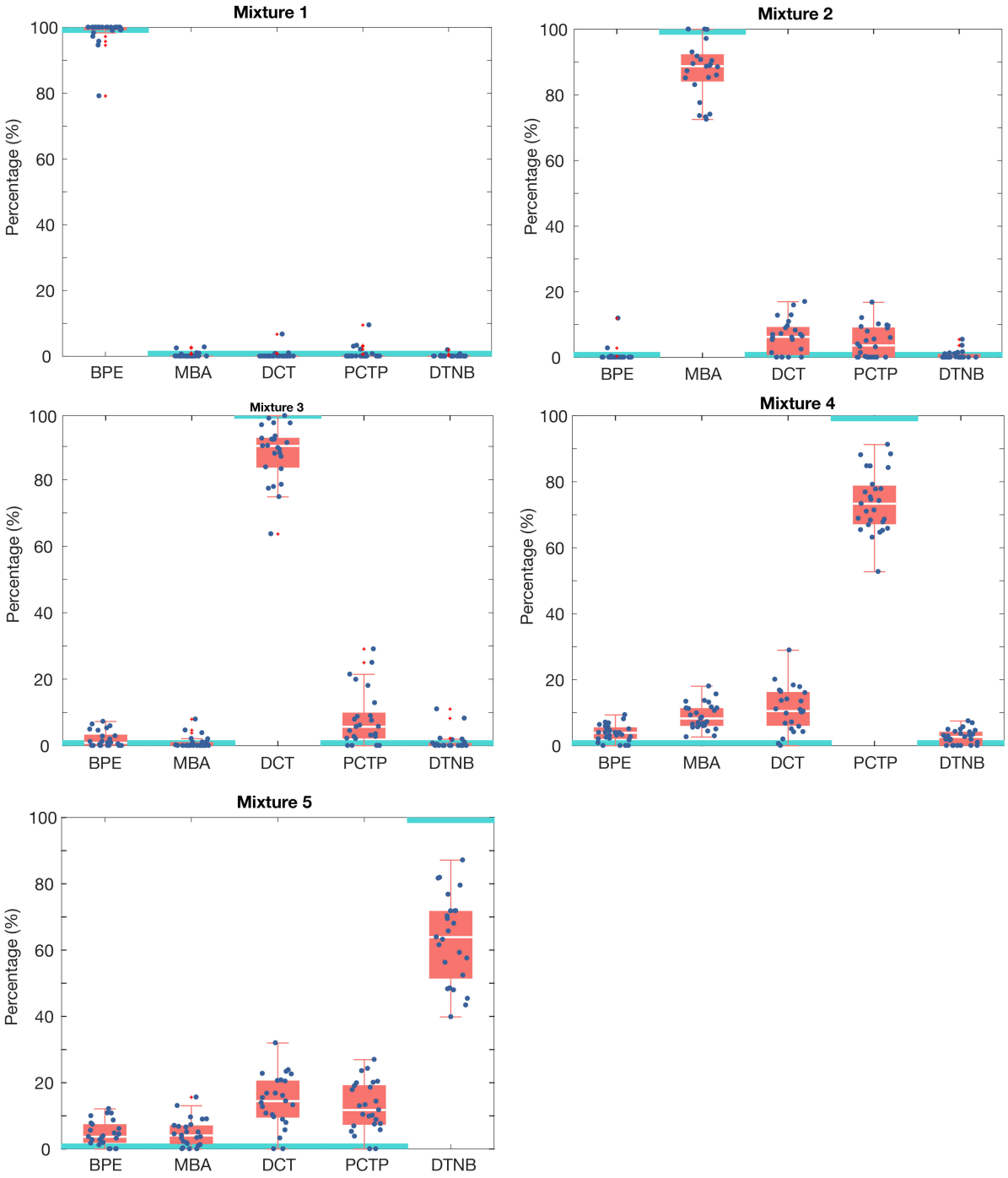


Figure S8: Box plots of Biomarker levels estimation for individual tests using least squares algorithm. Each data point represents the individual SERS intensities for a region of the test area. Red boxes show 50% of the data between the second (lower limit) and the third quartile (upper limit), and the median (white line). Whiskers indicate the value of the maximum and the minimum. SERS intensities were measured for 30 regions in a test area. Light blue boxes represent the real ratio of reporter in the mixture.

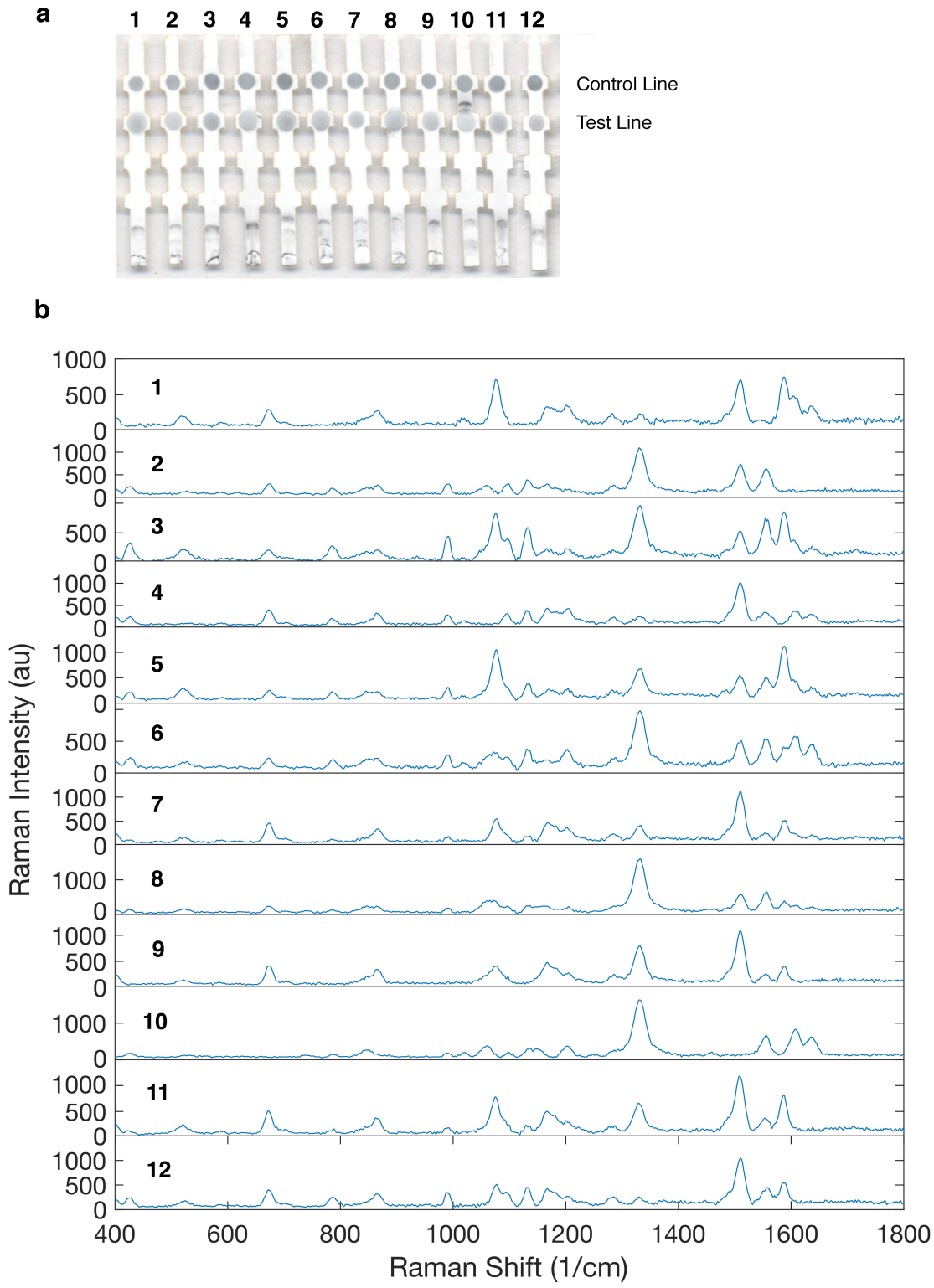
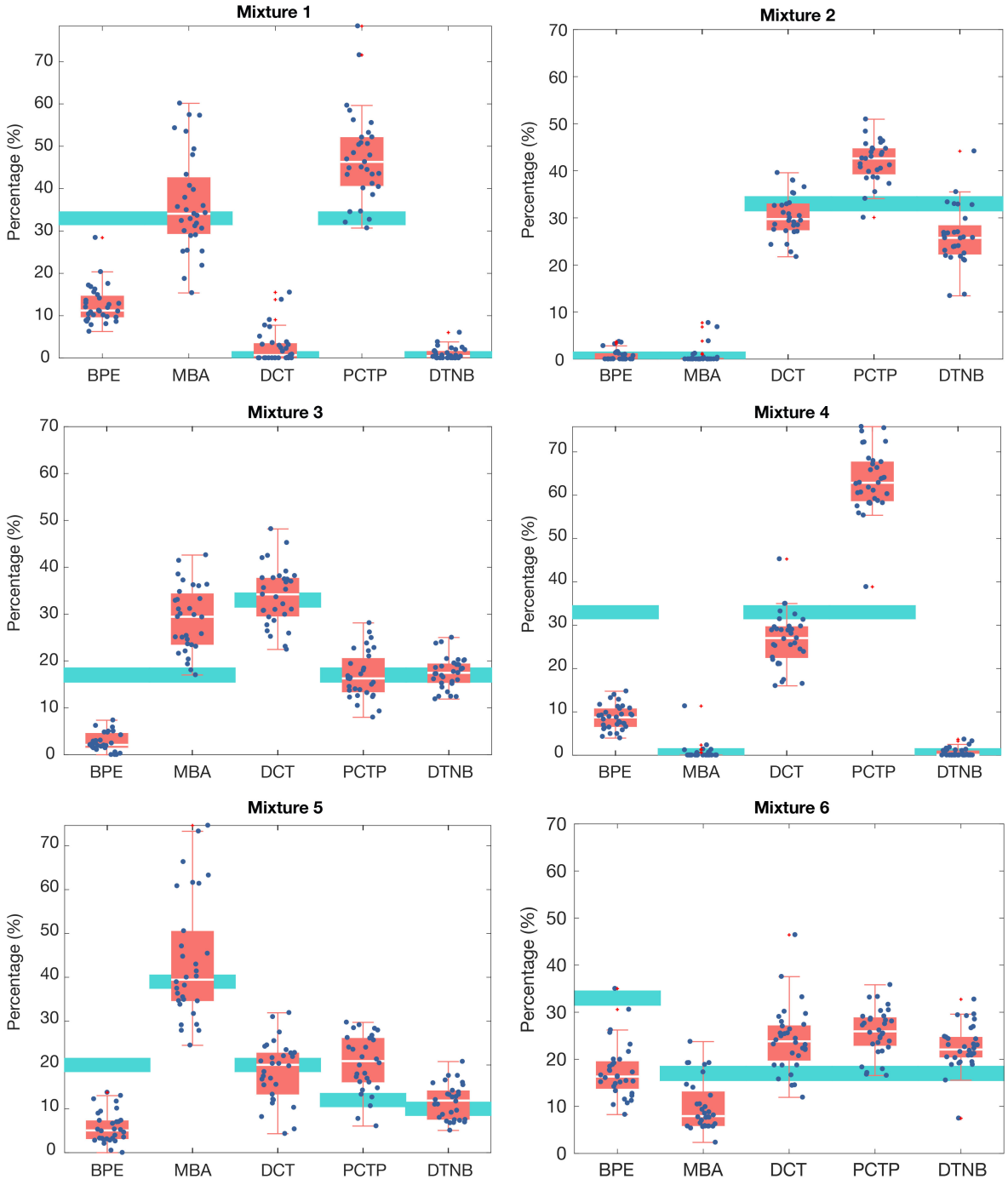


Figure S9: Sandwich immunoassay with the 12 mixtures prepared (testing set). a) Resulting strips from running the nanotags' mixtures (strips 1-12). b) SERS spectra of the 12 mixtures.


Mixture 1
Mixture 2
Mixture 3
Mixture 4
Mixture 5
Mixture 6
Percentage (%)
BPE
MBA
DCT
PCTP
DTNB

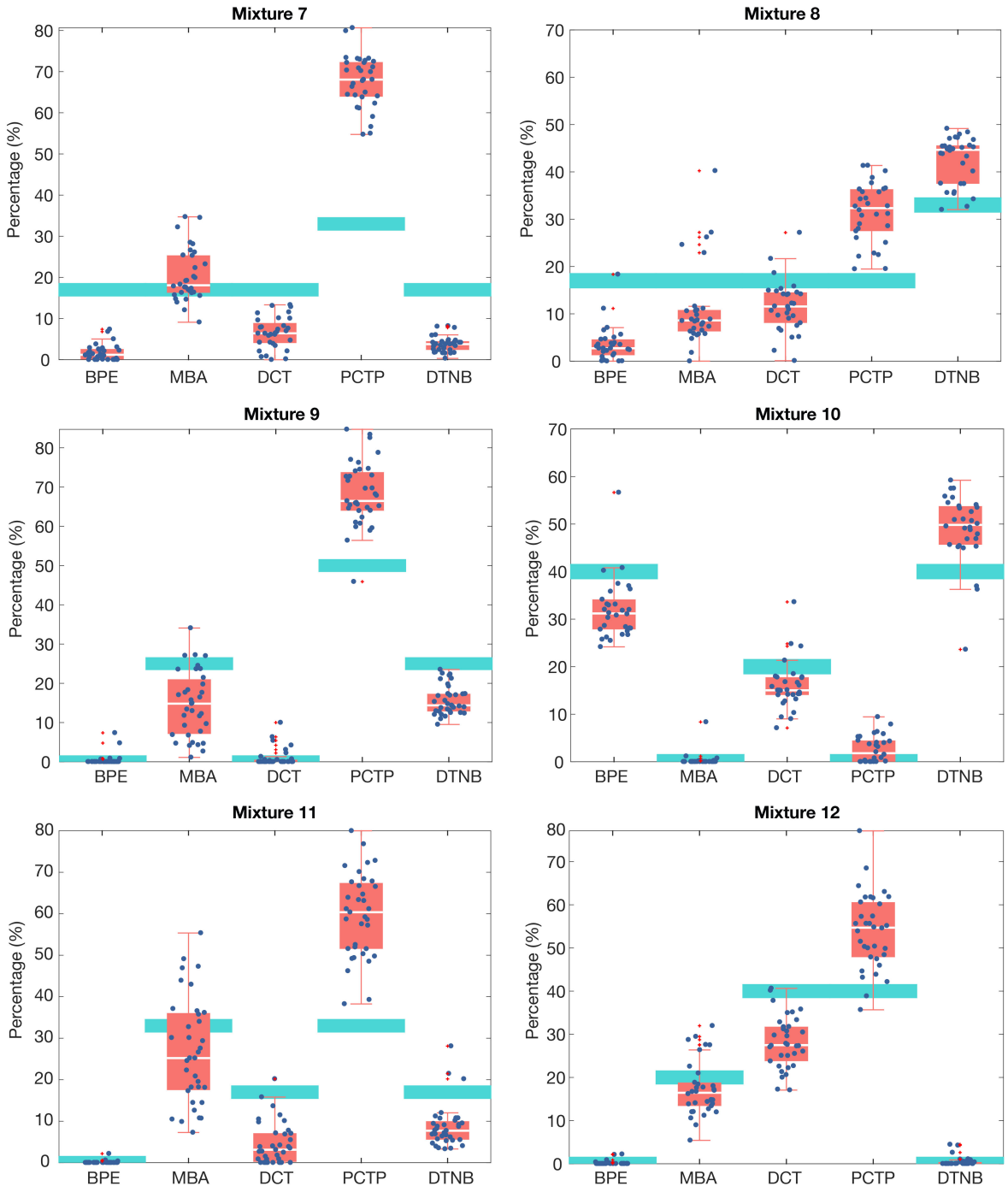


Figure S10. Box plots of Biomarker levels estimation in each mixture using least squares algorithm. Each data point represents the individual SERS intensities for a region of the test area. Red boxes show 50% of the data between the second (lower limit) and the third quartile (upper limit), and the median (white line). Whiskers indicate the value of the maximum and the minimum. SERS intensities were measured for 30 regions in a test area. Light blue boxes represent the real ratio of reporter in the mixtures (Table 1).

Comparison of SERS signal with and without LFA

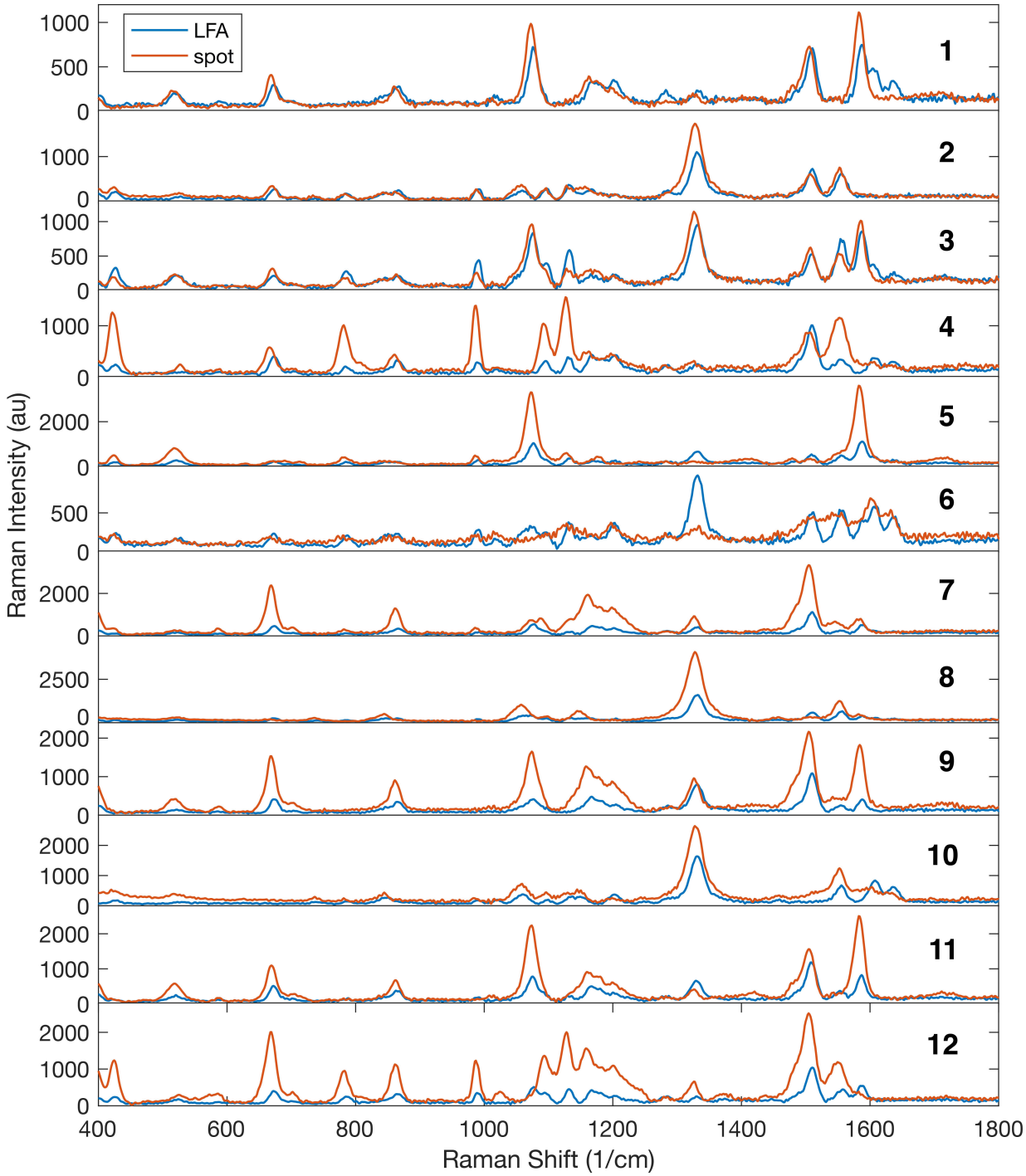
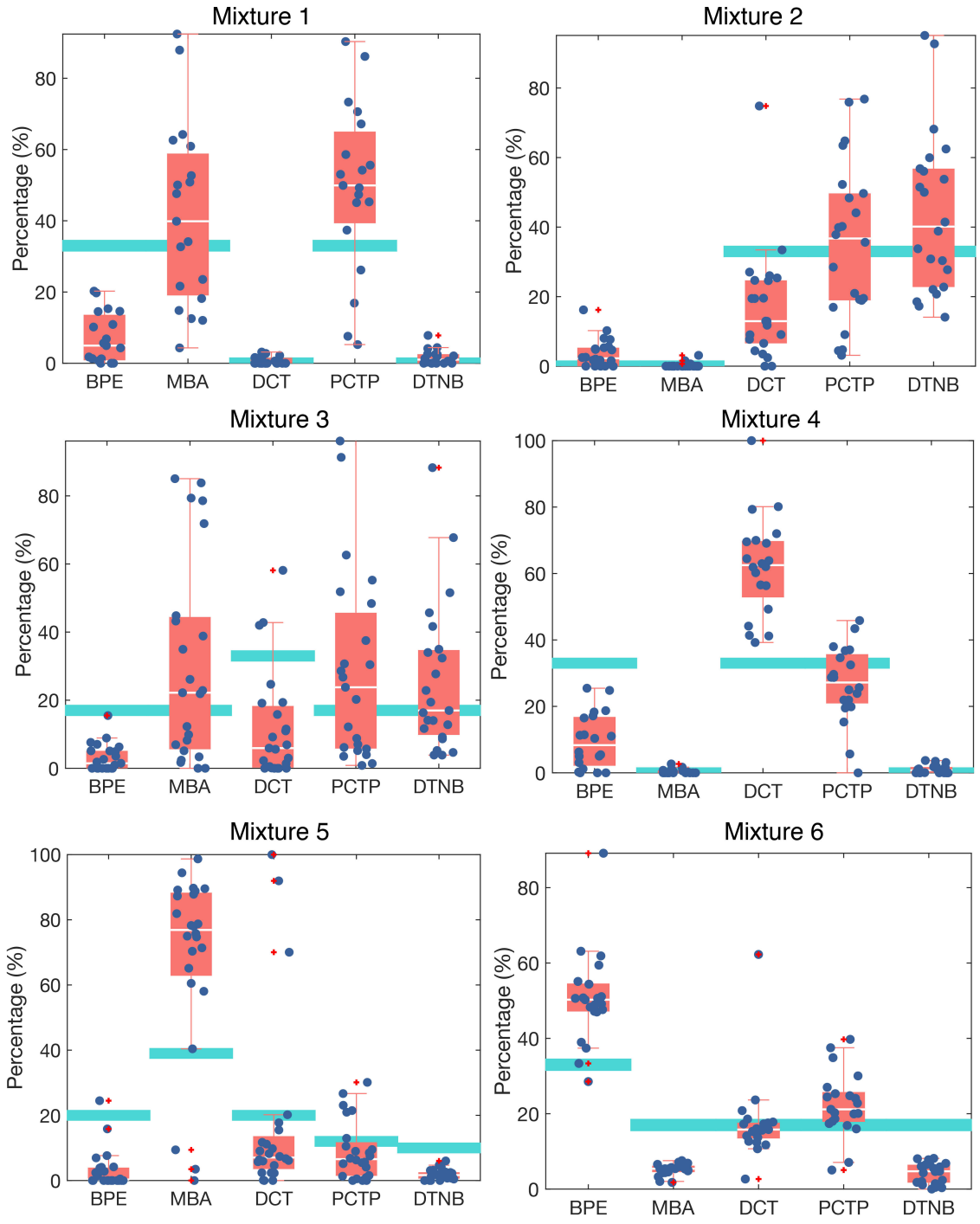


Figure S11: Comparison of SERS signal of the 12 samples prepared spotted (red) and on LFA (blue). Ratios of the samples are defined in Table 1.

Biomarkers' level estimation and mixture classification of spotted samples



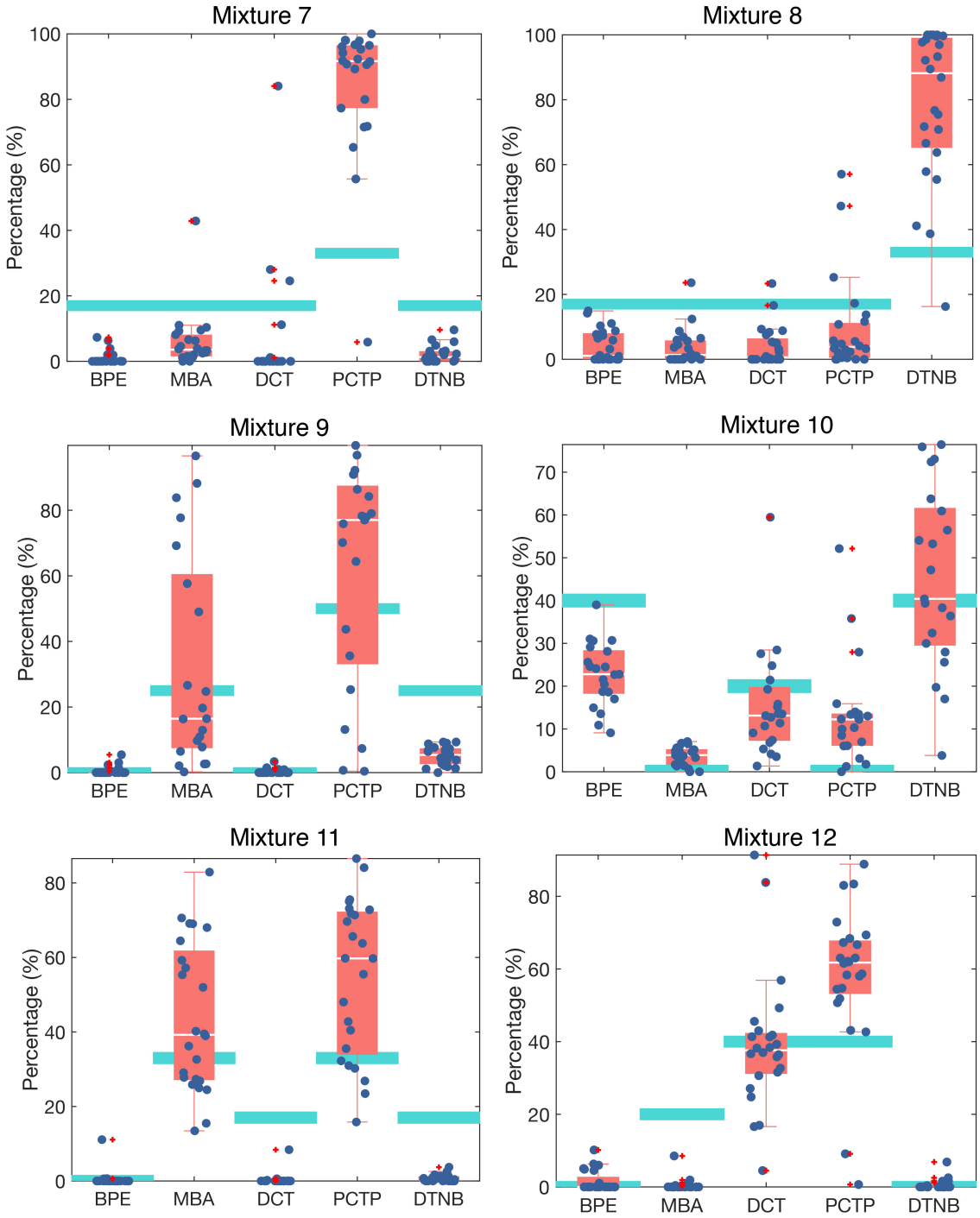


Figure S12: Box plots of Biomarker levels estimation in each spotted mixture using least squares algorithm. Each data point represents the individual SERS intensities for a region of the test area. Red boxes show 50% of the data between the second (lower limit) and the third quartile (upper limit), and the median (white line). Whiskers indicate the value of the maximum and the minimum. SERS intensities were measured for 30 regions in a test area. Light blue boxes represent the real ratio of reporter in the mixture (Table 1).

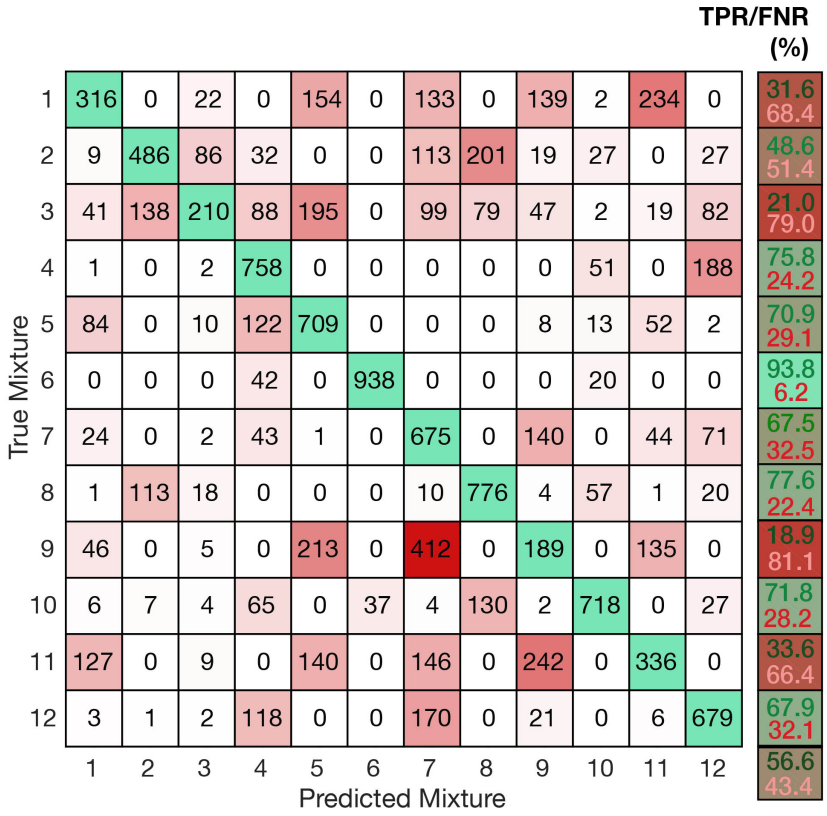


Figure S13: Confusion matrix of the 12 mixtures spotted (no LFA) and prepared as defined by Table 1 using LDA classifier.

References

1. Kah, J. C.-Y.; Zubieta, A.; Saavedra, R. A.; Hamad-Schifferli, K. Stability of Gold Nanorods Passivated with Amphiphilic Ligands. *Langmuir* **2012,** *28*, 8834-8844.