Electronic Supplementary Material (ESI) for Nanoscale.

Fluorescence-quenching Based Signal Amplification on Immunochromatography Test Strip for Dual-Mode Sensing Two Biomarkers of Breast Cancer

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Table S1. A comparison of the mFICTS and referenced immune assays for tumor markers.

Immune assays	Limits of detection		Referrence
	CEA (ng/mL)	C A153 (U/mL)	
Chemiluminescent immuneassay	0.65	0.8	[1]
Electrochemical immuneassay	—	0.3	[2]
Immunochromatography test strip	2	—	[3]
Immunochromatography test strip	0.35	—	[4]
Immunochromatography test strip	0.06	0.09	This work

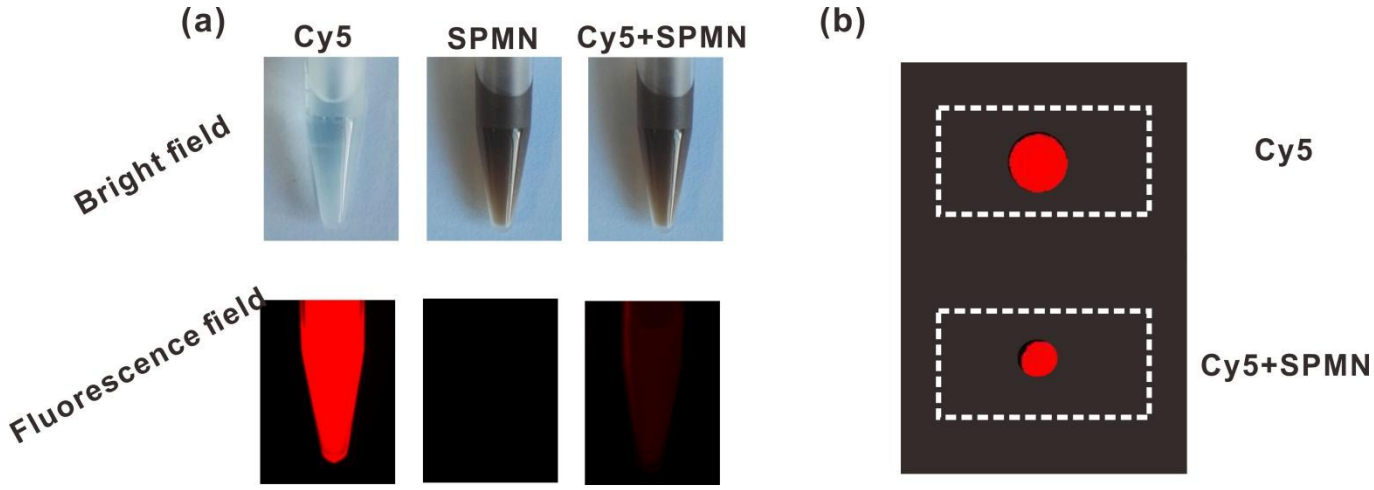

Figure S1. (a) Bright-field and fluorescence-field images of solutions containing Cy5; SPMN; and Cy5 with SPMN. (b) Fluorescence images of solutions containing Cy5 and Cy5 with SPMN on the nitrocellulose membrane.

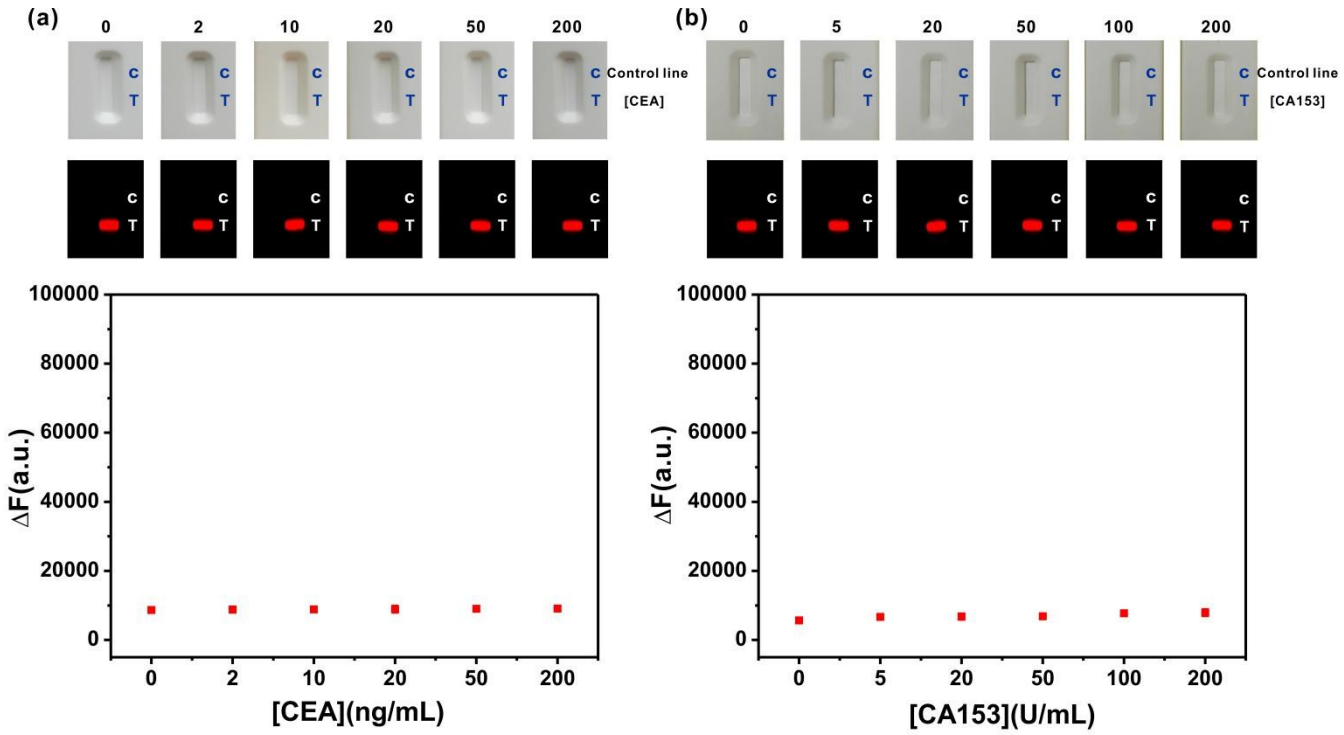

Figure S2. (a) Bright-field, fluorescence-field images and the fluorescent intensity change value of solutions without or with different concentrations of CEA antigen; (b) Bright-field, fluorescence-field images and the fluorescent intensity change value of solutions without or with different concentrations of CA153 antigen.

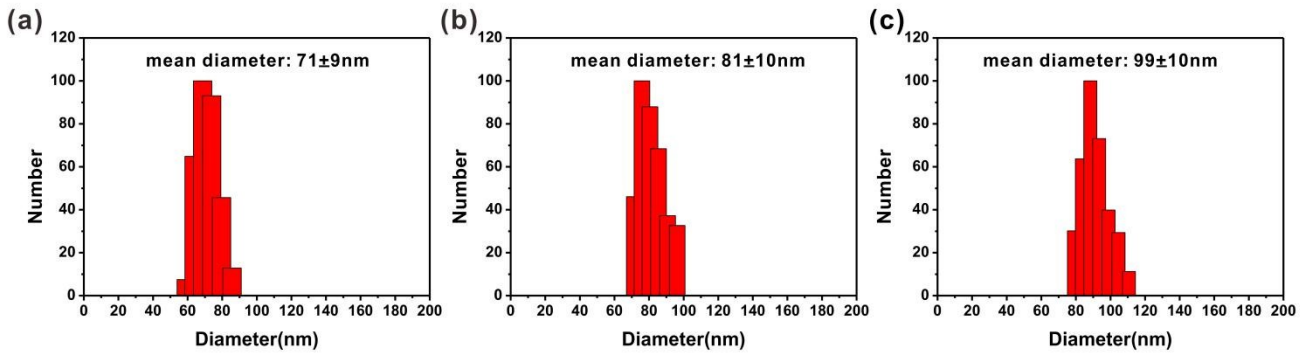

Figure S3. Size distribution of SPMN with different Fe_3O_4 MNPs content. (a) The Fe_3O_4 MNPs in one

SPMN with the concentration 3.6 mg/mL, (b) 6.0 mg/mL, (c) 8.4 mg/mL.

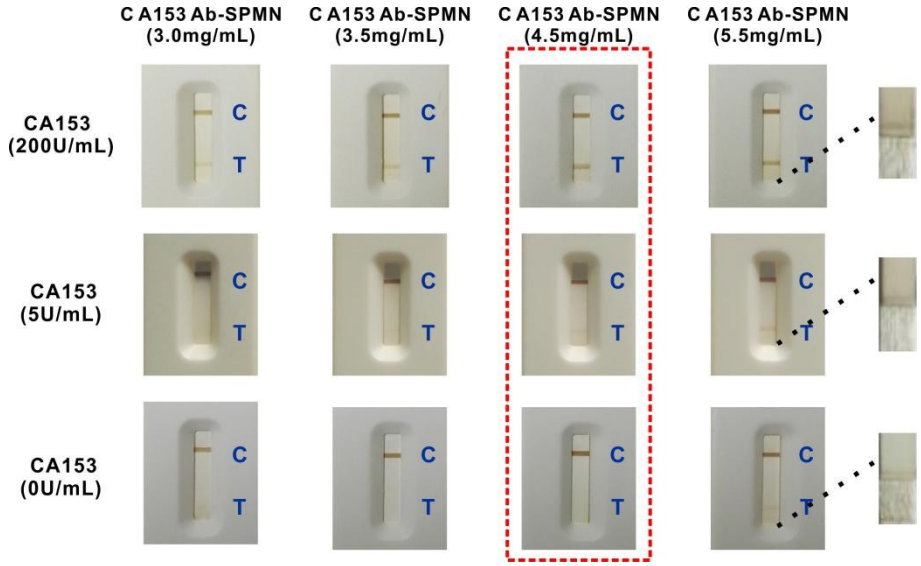

Figure S4. Colorimetric signal intensity under different concentrations of CA153 Ab-SPMN. The concentration of CA153 antigen were 0 U/mL, 5 U/mL and 200 U/mL respectively.

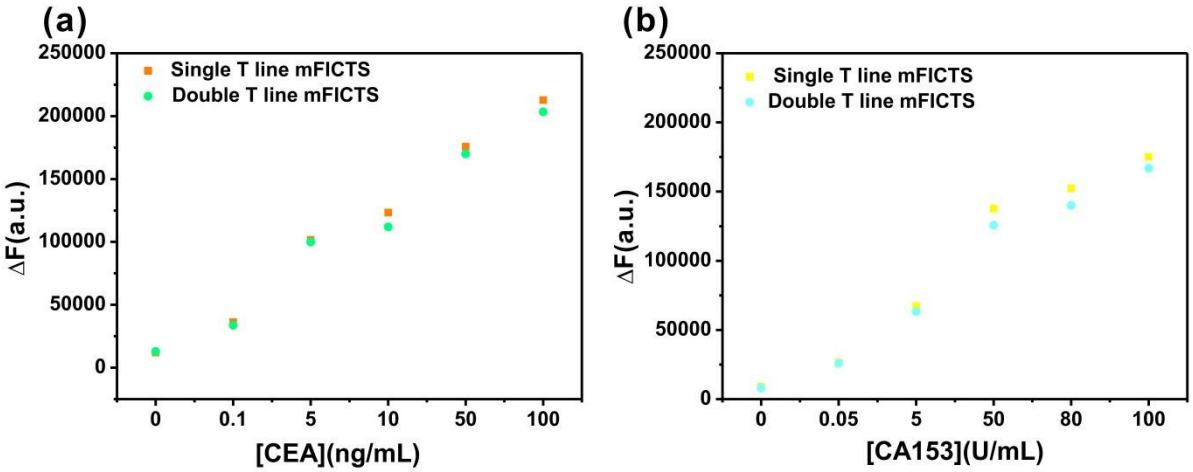

Figure S5. (a) The numerical simulation of single test line mFICTS and double test lines mFICTS for CEA. (b) The numerical simulation of single test line mFICTS and double test lines mFICTS for CA153.

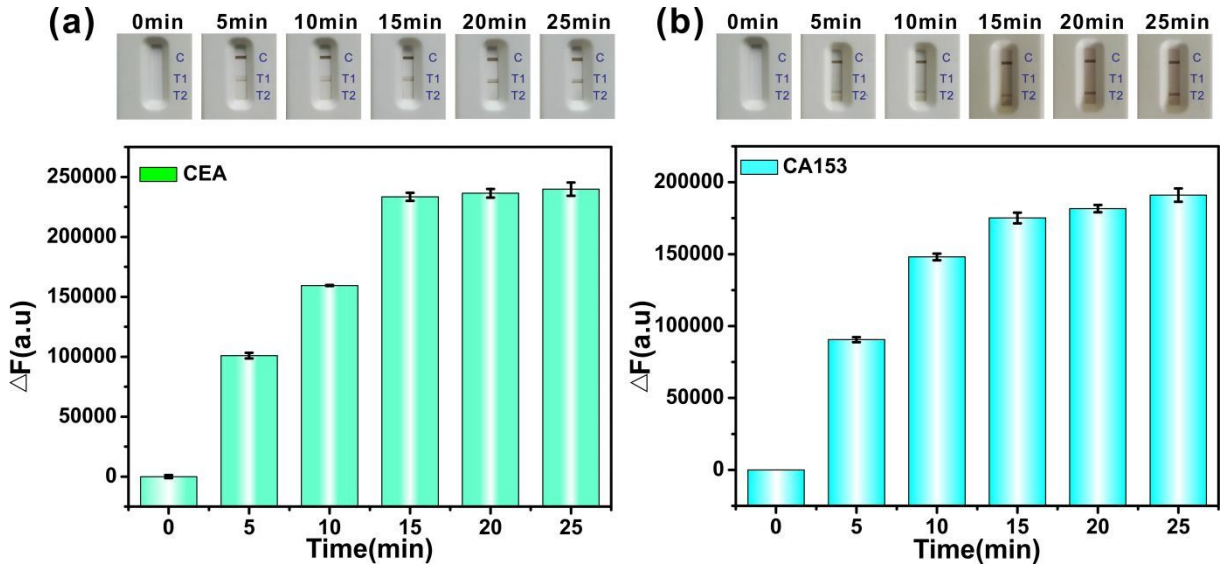

Figure S6. The effect test time of mFICTS. (a), (b) Qualitative detection time for CEA and CA153. The concentration of CEA antigen was 100 ng/mL and CA153 antigen was 100 U/mL.

Table S2. Clinical data analysis of patient serum by using the mFICTS.

	Sample size	Error Number	Error Rate		Sample size	Error Number	Error Rate
CA153 Positive Sample	32	2	6.25%	CEA Positive Sample	20	1	5%
CA153 Negative Sample	18	1	5.57%	CEA Negative Sample	30	2	6.67%
In All	50	3	6%	In All	50	3	6%

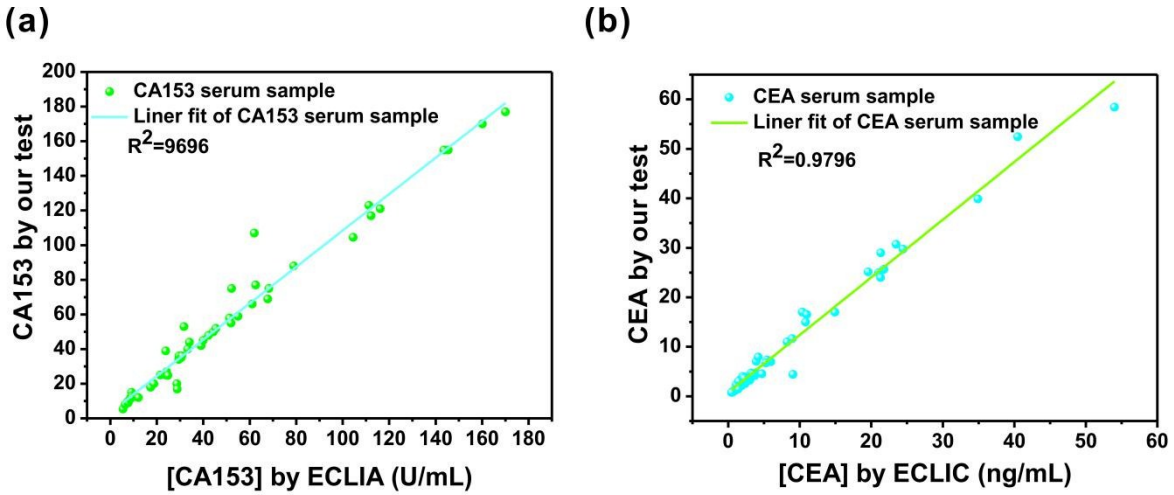

Figure S7. Linear fitting method for our test results and ECLIA detection results. (a) Linear fitting for CA153. (b) Linear fitting for CEA.

Figure S8. (a) The test strip which was cut into 3 mm preserved in the silver paper. (b) The sealed package of mFICTS. (c) Three repeat tests for the fluorescence intensity F0 before reaction at 0, 3, 7, 10, 14 days.

Rererence

[1] Z. Fu, Z. Yang, J. Tang, H. Liu, F. Yan and H. Ju, *ANAL CHEM*, 2007, **79**, 7376-7382.

[2] G. Wang, Y. Qing, J. Shan, F. Jin, R. Yuan and D. Wang, *MICROCHIM ACTA*, 2013, **180**, 651-657.

[3] C. Wang, F. Hou and Y. Ma, *BIOSENS BIOELECTRON*, 2015, **68**, 156-162.

[4] Z. Chen, R. Liang, X. Guo, J. Liang, Q. Deng, M. Li, T. An, T. Liu and Y. Wu, *BIOSENS BIOELECTRON*, 2017, **91**, 60-65.

Calculate the number of oil-soluble Fe_3O_4 NPs in one PLGA-Modified water-soluble super-paramagnetic nanosphere (SPMN).

$$Np=n_cV_1/V_2 \qquad \text{Eqn.1}$$

Np is the number of Fe in one Fe_3O_4 NPs, n_c is the number of Fe atom in one Fe_3O_4 cell; V_1 is the volume of Fe_3O_4 NPs; V_2 is the volume of Fe_3O_4 cell.

$$C_1=N_AC_{Fe}/(NpM_{Fe}) \qquad \text{Eqn.2}$$

C_1 is the particle concentration of Fe_3O_4 NPs; *NA* is the Avogadro Constant; C_{Fe} (μg/L) is the mass concentration of Fe atom which measured by the ICP-MS; M_{Fe} is the relative atomic mass of Fe atom.

$$C_2=C_m/[4/3\ \pi\ (d/2)^3\ \rho\ ] \qquad \text{Eqn.3}$$

C_2 is the particle concentration of PMAO-Modified water-soluble super-paramagnetic nanosphere (SPMN); *Cm* is the mass concentration of SPMN, ρ is the density of the SPMN.

$$N=C1/C2 \qquad \text{Eqn.4}$$

N is the number of oil-soluble Fe_3O_4 NPs in one PMAO-Modified water-soluble super-paramagnetic nanosphere (SPMN)

The quenching efficiency of the SPMN to the Cy5 molecules

$$n_{Cy5}=c(Ab)*V(Ab)*R_{Cy5/Ab}/M(Ab) \qquad \text{Eqn.5}$$

n_{Cy5} is the molar mass of Cy5; *c(Ab)* is the particle concentration of the capture antibody; *V(Ab)* is the reactive volume of capture antibody; $R_{Cy5/Ab}$ *is* the ratio of the Cy5 number with an antibdy; *M(Ab)* is the relative molecular mass of the antibody.

$$N_{quench}=n_{Cy5}*(F0-F)/(n_{SPMN}*F_0) \qquad \text{Eqn.6}$$

N_{quench} is the quenching capacity about one SPMN relative to Cy5 molecules. n_{SPMN} is the reactive molar mass of SPMN; *F0* is the fluorescence intensity before reaction; *F* is the fluorescence intensity after reaction.