Electronic Supplementary Material (ESI) for Analytical Methods.


**Electronic Supplementary Information**

***for***

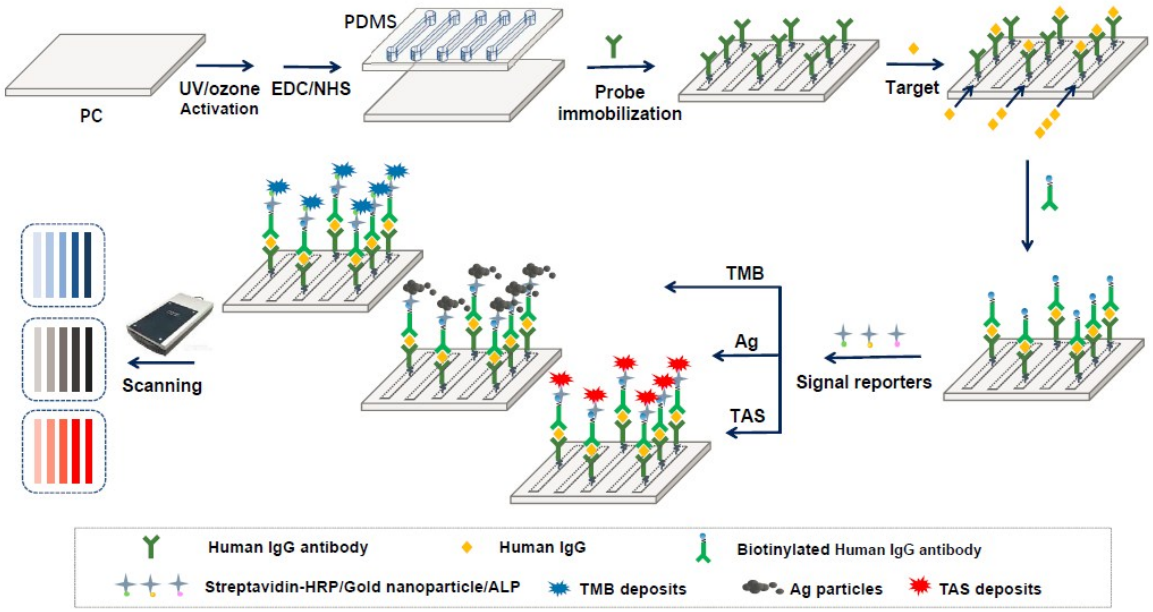
# Quantitative comparison of Three representative staining methods for the development of multichannel colorimetric biochips

**Mingxi Geng [a], Jiale He [a], Yunchao Li [a,*], Xiaoyi Gao [a] and Hua-Zhong Yu [b,*]**

[a] *Department of Chemistry, Beijing Normal University, Beijing 100875, P. R. China*

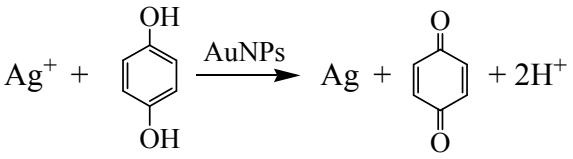
[b] *Department of Chemistry, Simon Fraser University, Burnaby, BC V5A 1S6, Canada*

[*] Corresponding authors: liyc@bnu.edu.cn (Y.L.); hogan_yu@sfu.ca (H.Y.)

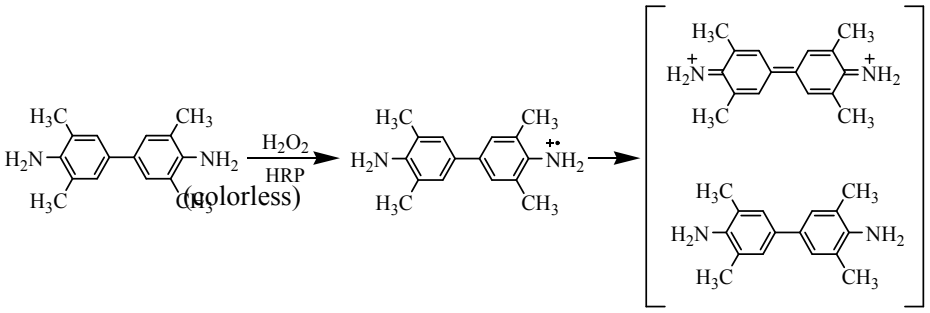



**Scheme S1** Schematic illustration of the fabrication and staining process of the immunochips designed for colorimetric detection of human IgG.

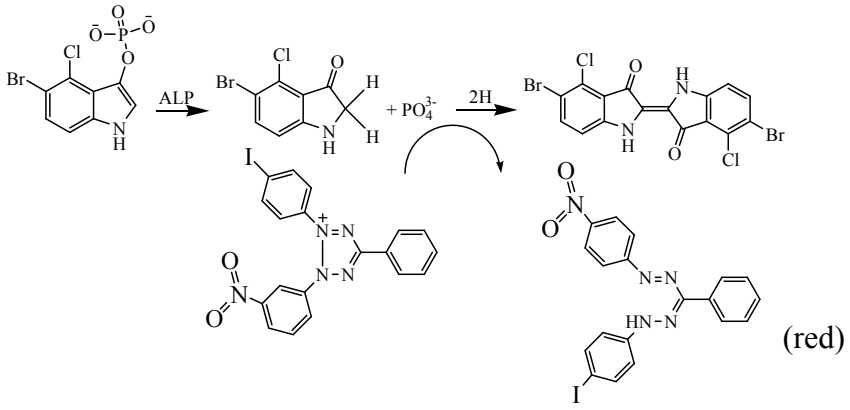
**Silver staining chromogenic reaction:**




**TMB staining chromogenic reaction:**




**TAS staining chromogenic reaction:**




**Scheme S2** Chemical reactions underlying the common staining methods for the development of colorimetric biochips.

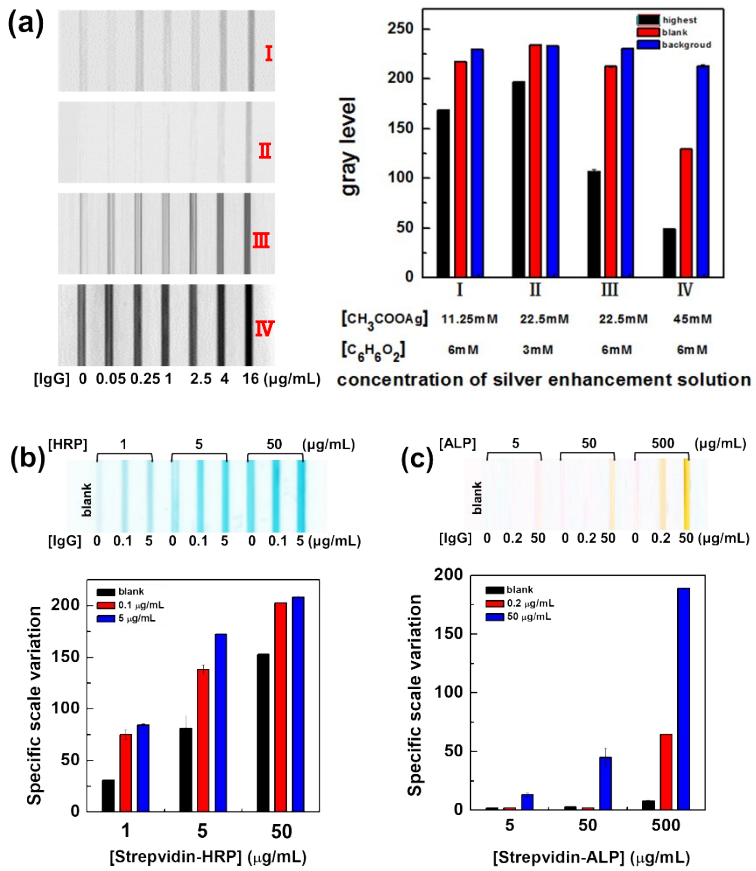



**Fig. S1** Optimization of the reaction conditions for the three common staining methods. Optical images and histograms to show the influence of the concentration of silver(I) acetate/hydroquinone (a), streptavidin-HRP (b), and streptavidin-ALP (c) on the signals developed by silver staining, TMB staining, and TAS staining, respectively.

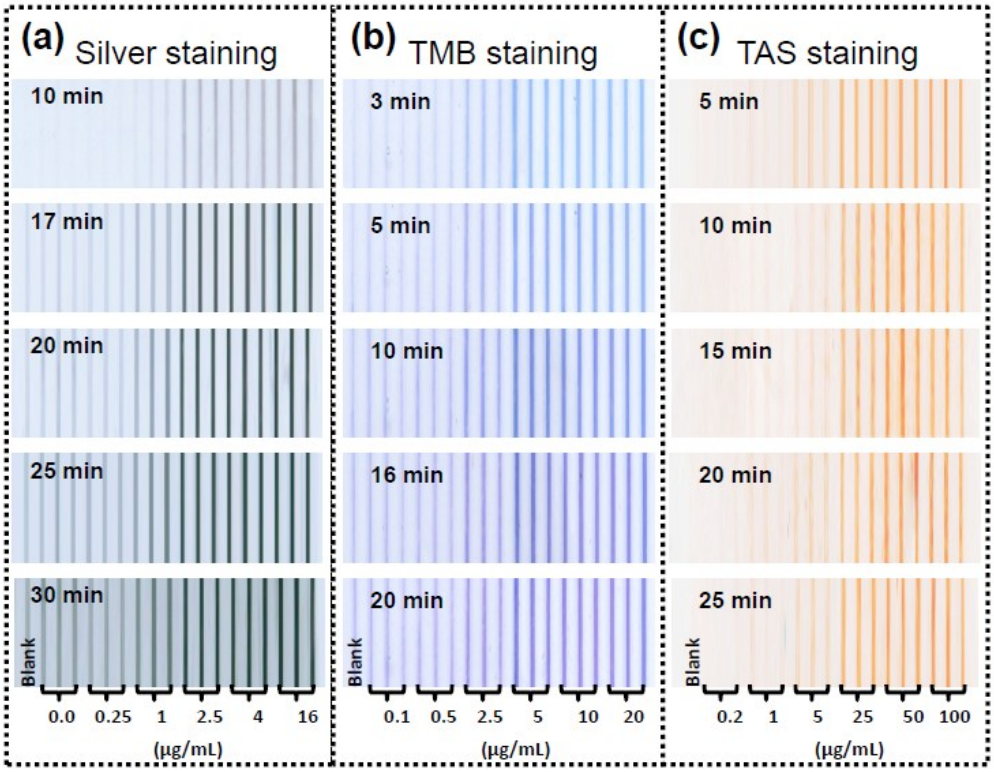



**Fig. S2** Optical images showing the assay development process of the three biochips developed by silver staining (a), TMB staining (b) and TAS staining (c), respectively.

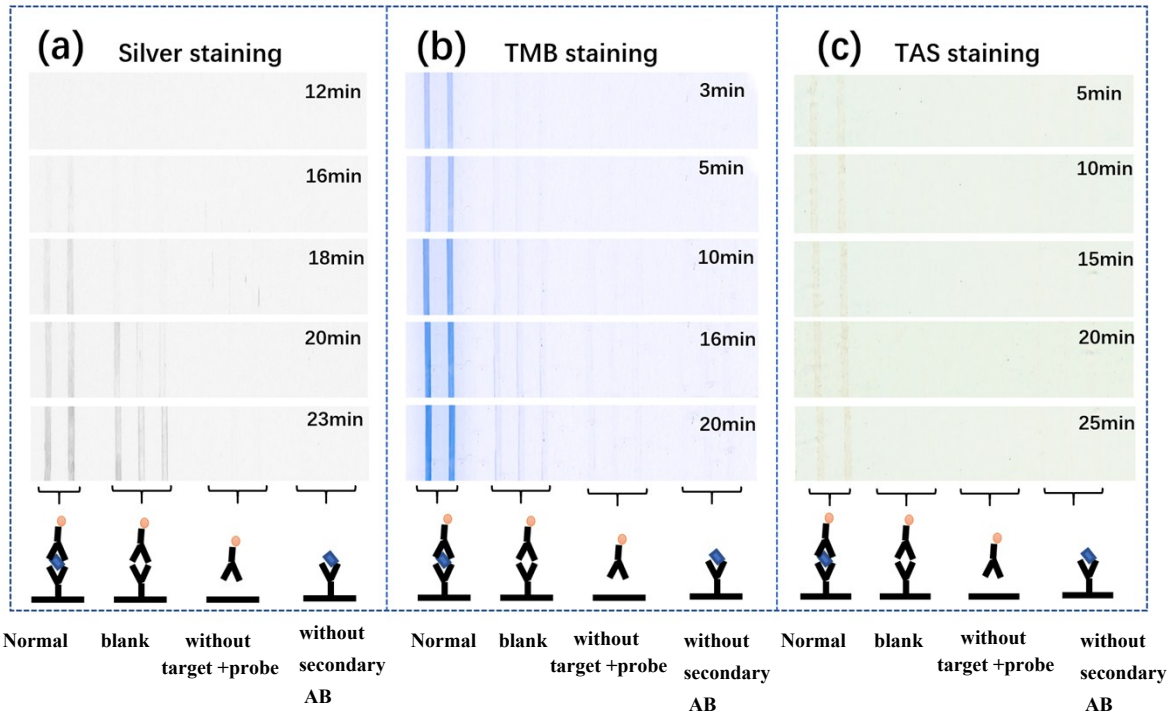



**Fig. S3** Optical images showing the the blank signal of the assay development process of the three biochips developed by silver staining (a), TMB staining (b) and TAS staining (c), respectively.

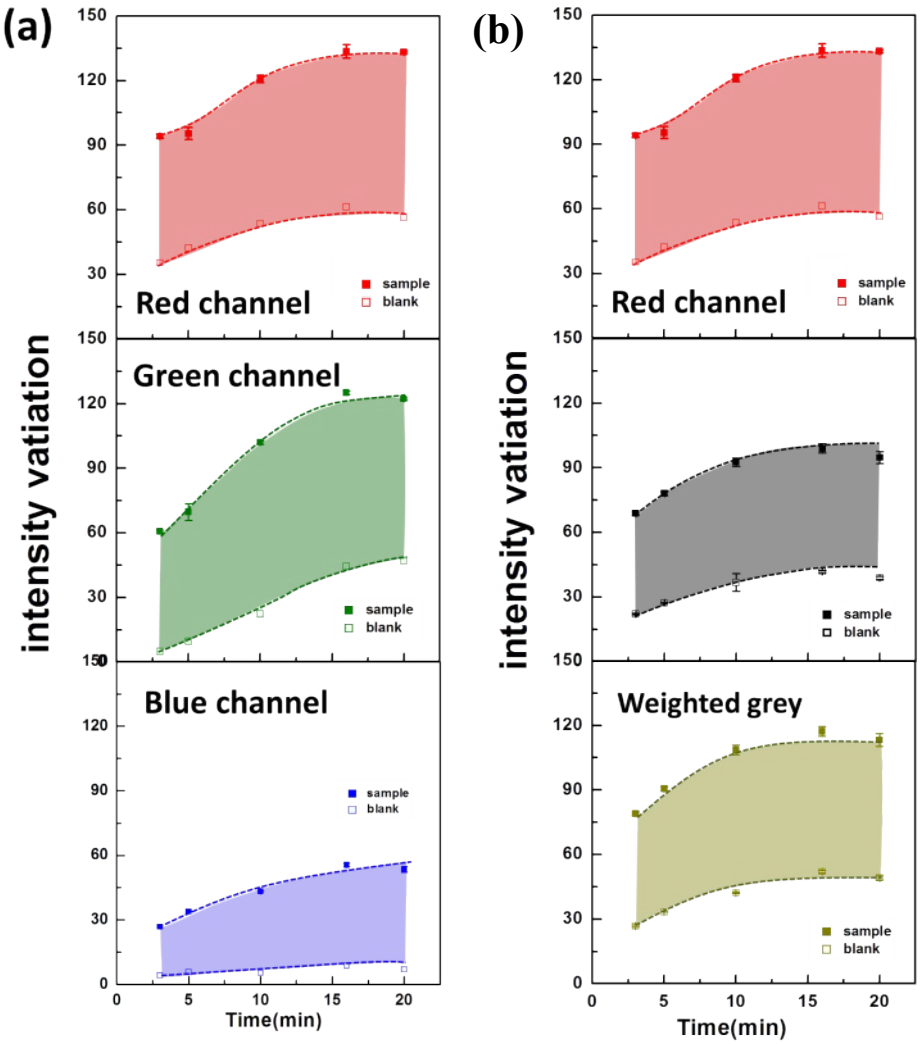



**Fig. S4** (a) Depicting the color variation of the strips developed by TMB staining with staining time by using the signal displaying in different color channels (i.e., red, green and blue channel), (b) comparison of the signal intensity in the most sensitive color channel (red) with that in two commonly-used grayscales, which are denoted as average grey, (R + G + B)/3, and weighted grey, R×0.299 + G×0.587 + B×0.114, respectively (where R, G, B are the corresponding pixel intensities in the red, green and blue channel).

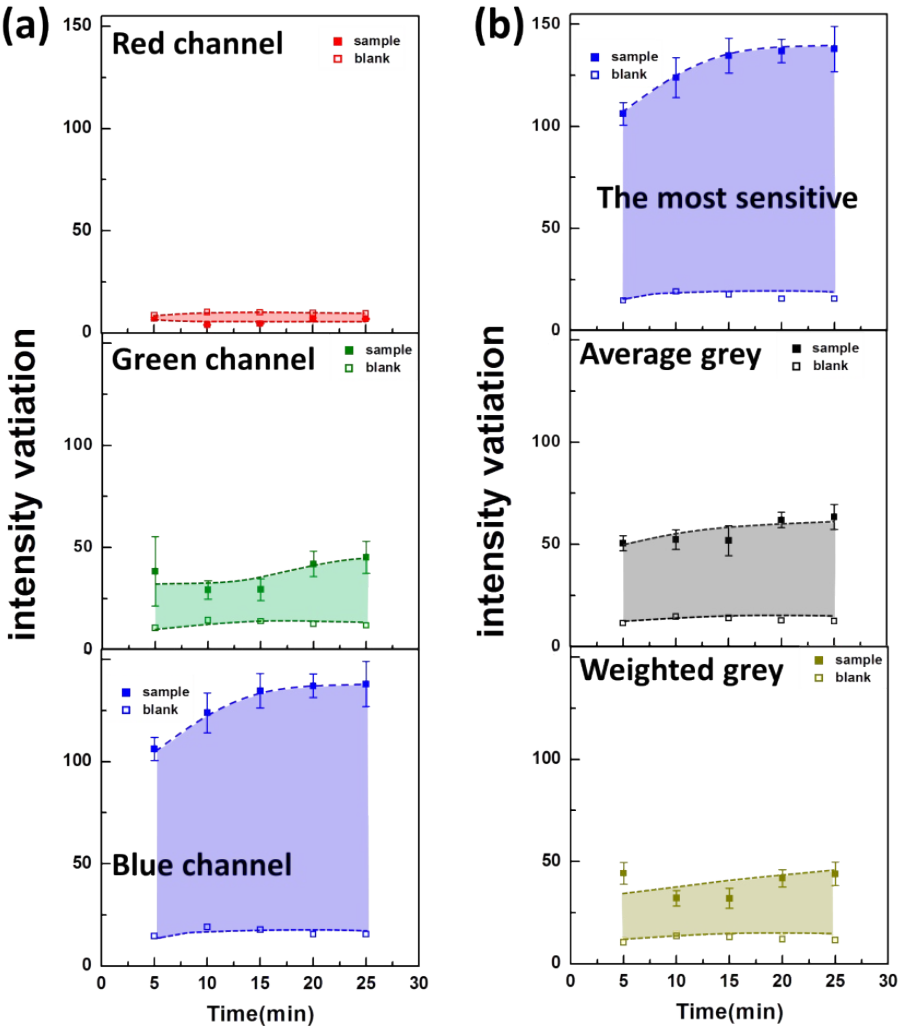



**Fig. S5** (a) Depicting the color intensity of the strips developed by TAS staining with staining time by using different color channels (i.e., red, green and blue channel), (b) comparison of the signal intensity reflected in the most sensitive color channel (blue) with that in two commonly-used grayscales (as described in Fig. S4).

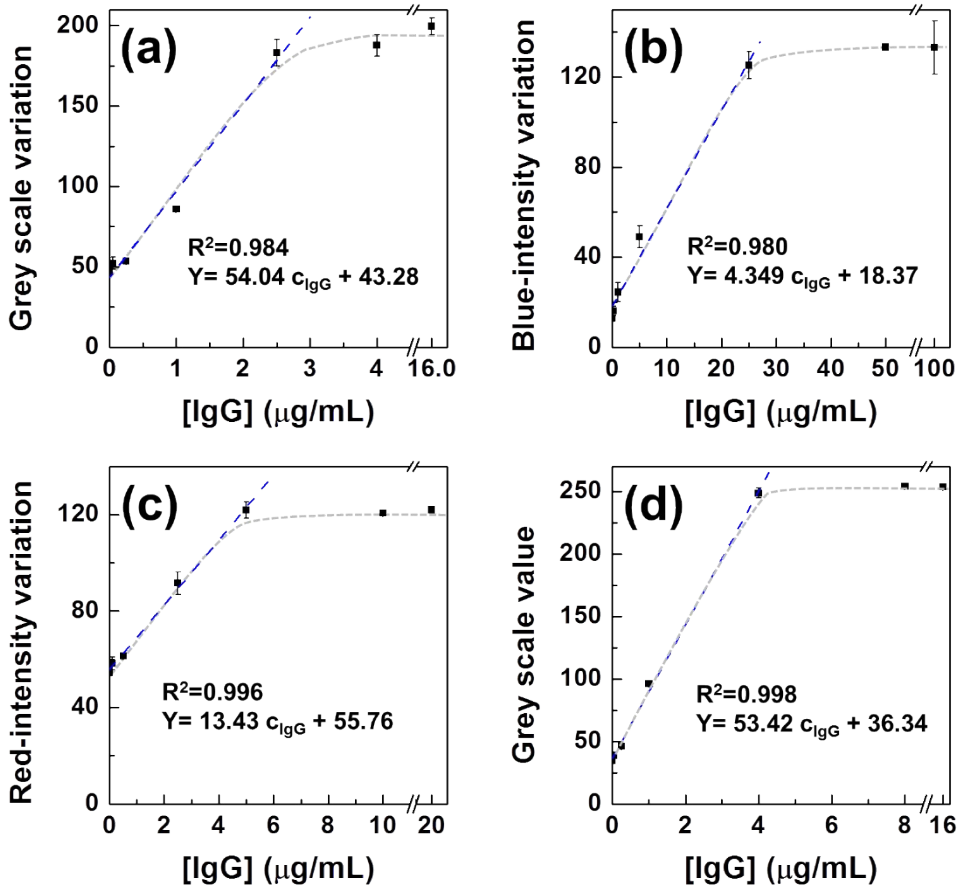



**Fig. S6** Linear response analysis of IgG immunoassay biochips developed by (a) silver staining, (b) TAS staining, (c) TMB staining, and (d) fluorescence method. The blue dashed line in each plot is the best fit (inset) to the experimental data.

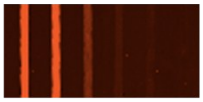
| | Silver staining | | | TMB staining | | | TAS staining | | | fluorescence method | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Fresh** |  | | |  | | |  | | |  | | |
| **6 months** |  | | |  | | |  | | |  | | |
| **[IgG] (μg/mL)** | 4 | 0.05 | 0 | 10 | 0.1 | 0 | 50 | 0.5 | 0 | 8 | 0.05 | 0 |
| **Remaining signal** | 97% | 81% | 74% | 87% | 73% | 64% | 76% | 72% | 57% | 89% | 93% | 97% |

**Fig. S7** Comparison of the signal stability of the stained biochips developed by silver staining, TMB staining and TAS staining before and after six month of storage in dark. For better comparision, the same chip with fluorescent labelling was also examned as a control.

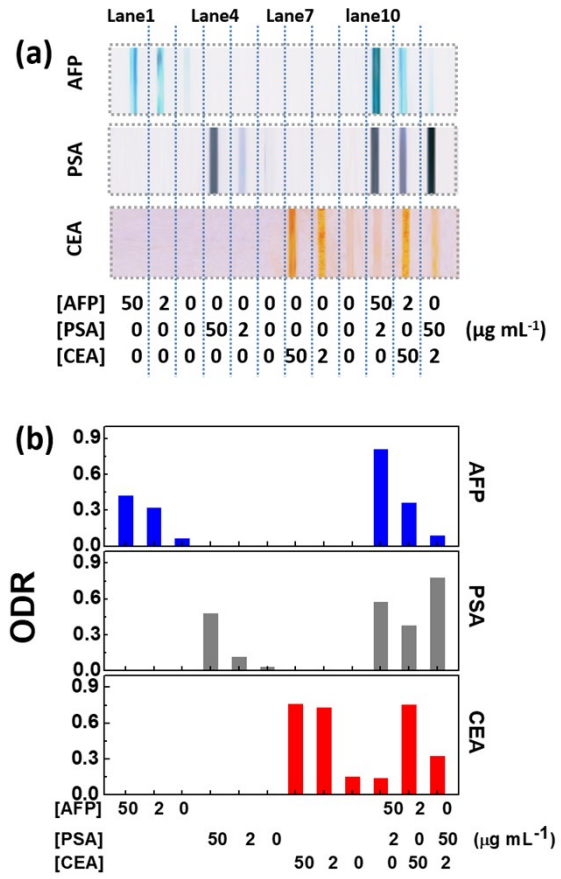



**Fig. S8** A multichannel colorimetric biochip for the detection of tumor markers (AFP, CEA, and PSA) in diluted human serum (a) Optical images showing the detection of individual marker and three makers on the same chip. (b) Histogram showing the signal of the three testing zones and concentration dependence.