

Supporting Information

An Automated microfluidic system based on V-groove chip for rapid immunohistochemistry

Lu Zhong^a, Hang Chen^b, Honglei Chen^b, Jun Peng^c, Zhi-Ling Zhang ^{a*}

^a College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, P. R. China

^b Department of Pathology, School of Basic Medical Sciences, Wuhan University, Wuhan 430071, P. R. China

^c Wuhan Pathologic Biotechnology Limited Company, Wuhan 430074, P. R. China

* Corresponding author

E-mail: zlzhang@whu.edu.cn (Z.-L. Zhang)

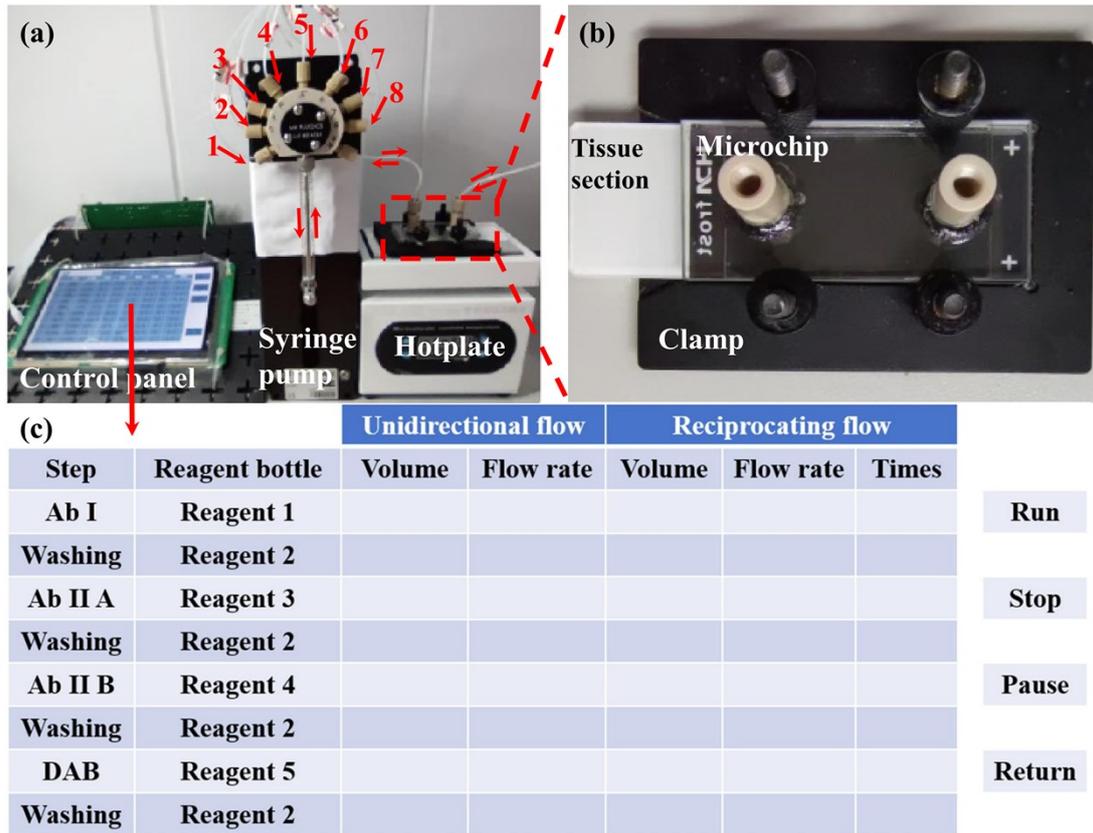


Fig. S1 Schematic diagram of the experimental setup for the microfluidic immunohistochemical staining system. (a) Overview of the experimental setup. (b) Microfluidic chip module. (c) Parameter setting interface (Reagent 1: Primary antibody reagent; Reagent 2: PBS buffer; Reagent 3: Secondary antibody reagent solution A (biotinylated secondary antibody); Reagent 4: Secondary antibody reagent solution B (streptavidin-HRP); Reagent 5: DAB chromogenic solution (3,3'-Diaminobenzidine)).

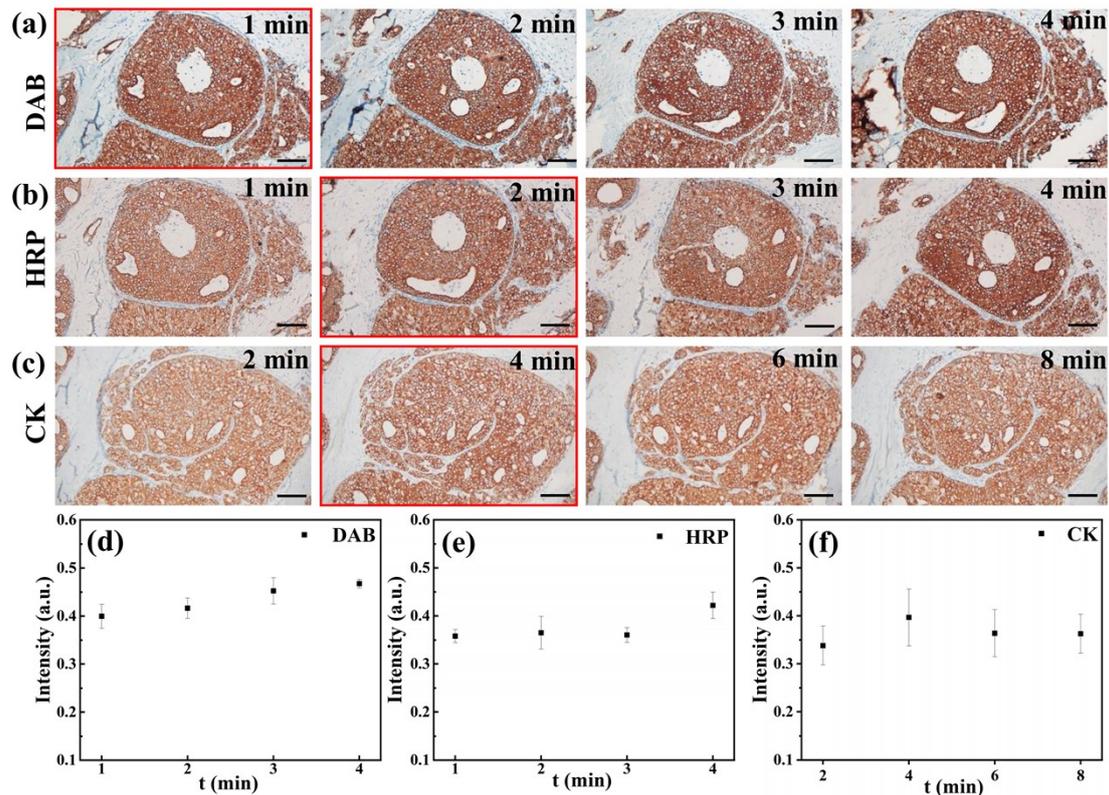


Fig. S2 Optimization of staining time conditions in microfluidic immunohistochemistry. The red box represents the optimal incubation time selected. (a) Optimization of DAB chromogenic time. (b) Optimization of horseradish peroxidase (HRP) incubation time. (c) Optimization of cytokeratin (CK) incubation time. (d–f) AOD values of staining under the corresponding conditions (n=3).

Table S1. Comparison of staining time between conventional and microfluidic immunohistochemistry methods.

Staining step	Conventional	V-chip
Primary antibody	40	4
Washing	9	0.3
Secondary antibody-I	15	2
Washing	9	0.3
Secondary antibody-II	20	2
Washing	9	0.3
DAB	10	1
Washing	9	0.3
Subtotal	122	11

Table S2. Comparison of staining results for five biomarkers between conventional and microfluidic immunohistochemistry methods.

Case	Biomarker	Staining outcome (conventional)	AOD (conventional)	Staining outcome (v-chip)	AOD (v-chip)
c1	CK	Positive (Pos)	0.301	Positive (Pos)	0.323
c2	CK	Positive (Pos)	0.339	Positive (Pos)	0.320
c3	CK	Positive (Pos)	0.227	Positive (Pos)	0.240
c4	CK	Positive (Pos)	0.229	Positive (Pos)	0.259
c5	CK	Positive (Pos)	0.344	Positive (Pos)	0.347
c6	CK	Positive (Pos)	0.326	Positive (Pos)	0.324
c7	CK	Positive (Pos)	0.348	Positive (Pos)	0.346
c8	CK	Positive (Pos)	0.348	Positive (Pos)	0.364
c9	CK	Positive (Pos)	0.297	Positive (Pos)	0.292
c10	CK	Positive (Pos)	0.396	Positive (Pos)	0.401
c11	CK	Negative (Neg)	0.000	Negative (Neg)	0.000
c12	CK	Positive (Pos)	0.257	Positive (Pos)	0.249
c13	CK	Positive (Pos)	0.322	Positive (Pos)	0.333
c14	CK	Positive (Pos)	0.306	Positive (Pos)	0.327
c15	Ki-67	Positive (+++)	0.417	Positive (+++)	0.424
c16	Ki-67	Positive(+)	0.336	Positive(+)	0.292
c17	Ki-67	Positive(+++)	0.528	Positive(+++)	0.488
c18	Ki-67	Positive(+++)	0.483	Positive(+++)	0.434
c19	Ki-67	Positive(+)	0.252	Positive(+)	0.275
c20	Ki-67	Positive(++)	0.322	Positive(++)	0.326
c21	Ki-67	Positive(+++)	0.421	Positive(+++)	0.403
c22	Ki-67	Positive(+++)	0.460	Positive(+++)	0.434
c23	Ki-67	Positive(+++)	0.412	Positive(+++)	0.429
c24	Ki-67	Positive (+++)	0.447	Positive (+++)	0.462
c25	Ki-67	Positive (+++)	0.405	Positive (+++)	0.415
c26	Her-2	Positive (+++)	0.389	Positive (+++)	0.383
c27	Her2	Positive (+++)	0.386	Positive (+++)	0.364
c28	Her-2	Negative(-)	0.000	Negative(-)	0.000
c29	Her-2	Positive(+)	0.188	Positive(+)	0.192
c30	Her-2	Positive (+++)	0.482	Positive (+++)	0.434
c31	CK5/6	Positive (Pos)	0.408	Positive (Pos)	0.409
c32	CK5/6	Positive (Pos)	0.295	Positive (Pos)	0.280
c33	CK5/6	Positive (Pos)	0.336	Positive (Pos)	0.343
c34	CK5/6	Positive (Pos)	0.357	Positive (Pos)	0.338
c35	CK5/6	Positive (Pos)	0.320	Positive (Pos)	0.325
c36	CD3	Positive (Pos)	0.464	Positive (Pos)	0.448
c37	CD3	Positive (Pos)	0.329	Positive (Pos)	0.307
c38	CD3	Positive (Pos)	0.319	Positive (Pos)	0.323
c39	CD3	Positive (Pos)	0.399	Positive (Pos)	0.455
c40	CD3	Positive (Pos)	0.381	Positive (Pos)	0.388