Supplementary Information

An integrated microfluidic system for rapid, automatic and high-throughput staining of clinical tissue samples for diagnosis of ovarian cancer

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Table S1. Aptamer sequence specific to an ovarian cancer gene and its dissociation constant (Kd) [1].

Name of aptamer	Aptamer (5'-3') sequence	Kd (nM)
Tx-01	ACAGCACCACAGACCATCAAATTA GGAAAATCATGACGGGGTGGAACC GAGGGGGTGTTTGTCTTCCTGCC	53.8±14.9

Table S2. Experimental procedure for on-chip, aptamer-based immuno-histochemistry (IHC). A similar outcome could be achieved by replacing the aptamer with an antibody (*i.e.*, antibody-based IHC). For each step, the reagent volume was 490 µL. Pressures and frequencies in the "Operating conditions" column refer to the applied gauge pressure and micro-stirrer activity, respectively. RT=room temperature.

Step	Procedure	Operating conditions
1	Transport dewaxing buffer into micro-stirrer chamber	95°C for 20 min
	Mix dewaxing buffer with tissue samples for 20 min	-33.3 kPa and 1 Hz
2	Transport PBS into micro-stirrer chamber	1 min x 3
	Wash tissue samples with PBS for 3 min	-33.3 kPa and 1 Hz
3	Transport 3% H_2O_2 into micro-stirrer chamber	RT for 10 min
	Mix H_2O_2 with tissue samples for 10 min	-33.3 kPa and 1 Hz
4	Transport PBS into micro-stirrer chamber	1 min x 3
	Wash tissue samples with PBS for 3 min	-33.3 kPa and 1 Hz
5	Transport avidin into micro-stirrer chamber	RT for 10 min
	Mix avidin with tissue samples for 10 min	-33.3 kPa and 1 Hz
6	Transport PBS into micro-stirrer chamber	1 min x 3
	Wash tissue samples with PBS for 3 min	-33.3 kPa and 1 Hz
7	Transport biotin into micro-stirrer chamber	RT for 10 min
	Mix biotin with tissue samples for 10 min	-33.3 kPa and 1 Hz
8	Transport PBS into micro-stirrer chamber	1 min x 3
	Wash tissue samples with PBS for 3 min	-33.3 kPa and 1 Hz
9	Transport Tx-01-biotin into micro-stirrer chamber	RT for 30 min
	Mix Tx-01-biotin with tissue samples for 30 min	-33.3 kPa and 1 Hz
10	Transport PBS into micro-stirrer chamber	1 min x 3
	Wash tissue samples with PBS for 3 min	-33.3 kPa and 1 Hz
11	Transport streptavidin-HRP into micro-stirrer chamber	RT for 15 min
	Mix streptavidin-HRP with tissue samples for 15 min	-33.3 kPa and 1 Hz
12	Transport PBS into micro-stirrer chamber	1 min x 3
	Wash tissue samples with PBS for 3 min	-33.3 kPa and 1 Hz
13	Transport DAB substrate into micro-stirrer chamber	RT for 1 min
	Mix DAB with tissue samples for 1 min	-33.3 kPa and 1 Hz
14	Transport PBS into micro-stirrer chamber	1 min x 3
	Wash tissue samples with PBS for 3 min	-33.3 kPa and 1 Hz
15	Transport hematoxylin into micro-stirrer chamber	RT for 5 min
	Mix hematoxylin with tissue samples for 15 min	-33.3 kPa and 1 Hz
16	Transport PBS into micro-stirrer chamber	1 min x 3

	Wash tissue samples with PBS for 3 min	-33.3 kPa and 1 Hz

Table S3. Experimental procedures for on-chip, aptamer-based fluorescent staining. For all steps, the reagent volume was 490 μL. Pressures and frequencies in the "Operating conditions" column refer to the applied gauge pressure and micro-stirrer activity, respectively. RT=room temperature.

Step	Procedure	Operating conditions
1	Transport dewaxing buffer into micro-stirrer chamber	95°C for 20 min
	Mix TrueBlack with tissue samples for 10 min	-33.3 kPa and 1 Hz
2	Transport PBS into micro-stirrer chamber	1 min x 3
	Wash tissue samples with PBS for 3 min	-33.3 kPa and 1 Hz
3	Transport TrueBlack into micro-stirrer chamber	RT for 10 min
	Mix TrueBlack with tissue samples for 10 min	-33.3 kPa and 1 Hz
4	Transport PBS into micro-stirrer chamber	1 min x 3
	Wash tissue samples with PBS for 3 min	-33.3 kPa and 1 Hz
5	Transport Tx-01-cy3 into micro-stirrer chamber	RT for 30 min
	Mix Tx-01-cy3 with tissue samples for 30 min	-33.3 kPa and 1 Hz
6	Transport PBS into micro-stirrer chamber	1 min x 3
	Wash tissue samples with PBS for 3 min	-33.3 kPa and 1 Hz
7	Transport Hoechst into micro-stirrer chamber	RT for 3 min
	Mix Hoechst with tissue samples for 3 min	-33.3 kPa and 1 Hz
8	Transport PBS into micro-stirrer chamber	1 min x 3
	Wash tissue samples with PBS for 3 min	-33.3 kPa and 1 Hz

Table S4. Pumping volume of 10 micro-pumps in each chamber. Error terms represent standard deviation.

Chamber number	Transport volume (ul)
1	490.1±3.1
2	489.6±1.1
3	491.2±0.8
4	488.6±1.8
5	487.5±3.4
6	490.5±2.9
7	487.3±3.1
8	491.1±2.5
9	488.2±3.1
10	490.1±1.6

Table S5. Temperature measurements at five points on the chip (see. Fig. S5.).

Time (min)	A	B	C	D	E
5	107.3	107.5	105.8	108.6	105.8
10	108.1	107.9	106.5	108.8	107.2
15	108.3	107.6	106.1	107.5	107.4
20	108.3	108.1	107.6	106.8	107.1

Table S6: H-score measurements for normal clinical and cancerous samples.

Sample number	**H-score**
1-cancerous	358
2-cancerous	337
3-cancerous	325
4-cancerous	352
5-cancerous	367
6-healthy	100

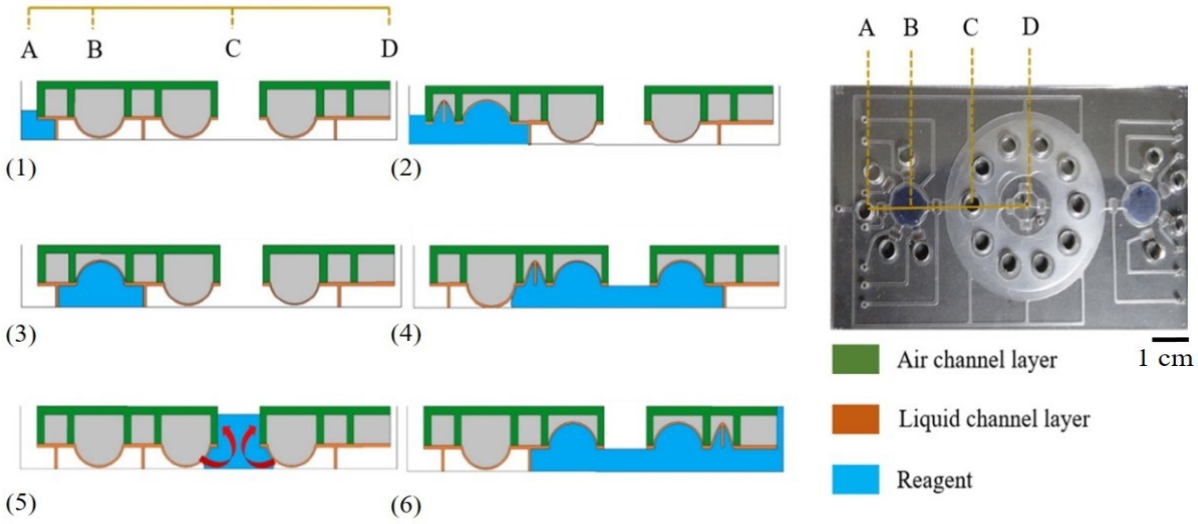


Figure S1(a)

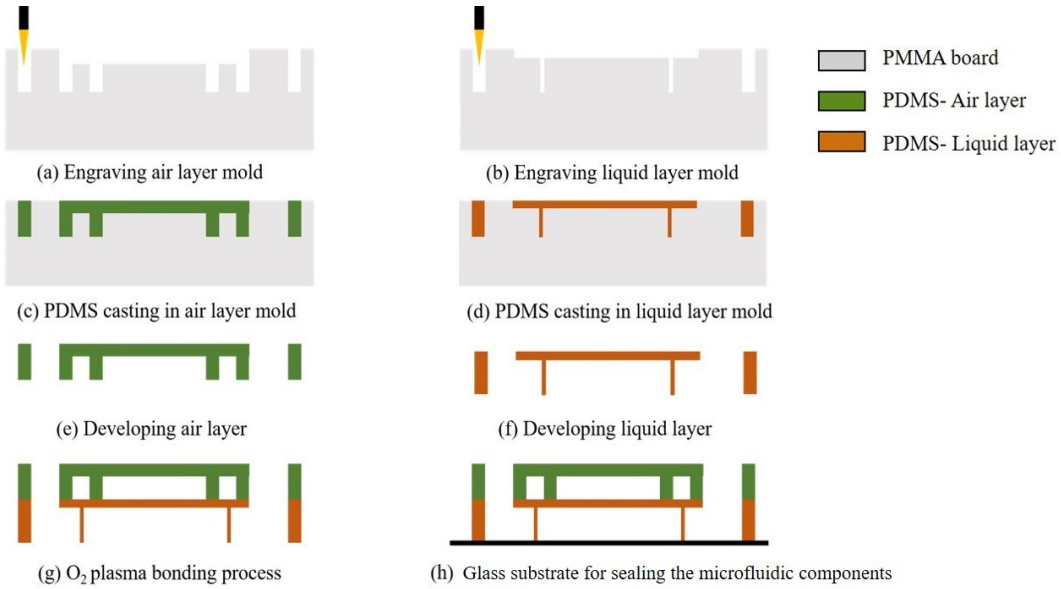


Figure S1(b)

Figure S1. (a). The working principle of the micro-components of the microfluidic chip. (1) All components were switched off at the initial stage, and the reagents were stored in the storage chambers. (2) Turning on the left 1) microvalve and 2) micro-pump resulted in liquid suction. (3) Turning off the left microvalve moved reagents to the micro-pump. (4) Turning on the right microvalve and left micro-pump transported

liquid into the micro-stir chamber. (5) Reagents were stirred in the micro-stir chamber by switching on/off the lateral micro-pump. (6) Switching on the right and switching off the 1) left microvalve and 2) micro-pump resulting in the pumping of liquid into the right-side waste channel. **(b).** Schematic diagram of microfluidic chip fabrication.

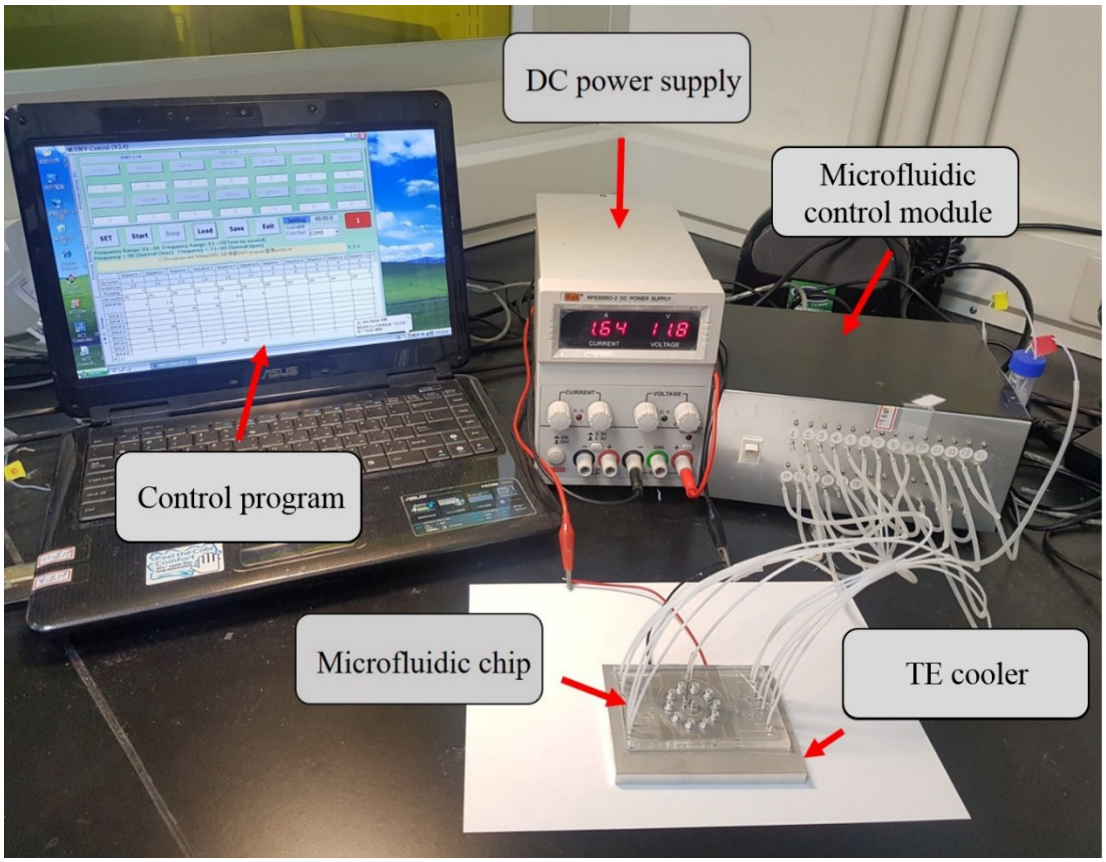


Figure S2. Experimental setup for on-chip clinical tissue staining. Upon integrating a thermoelectric (TE) cooler, a microfluidic control module, a DC power supply, and a computer, the entire protocol discussed in the main text could be automated.

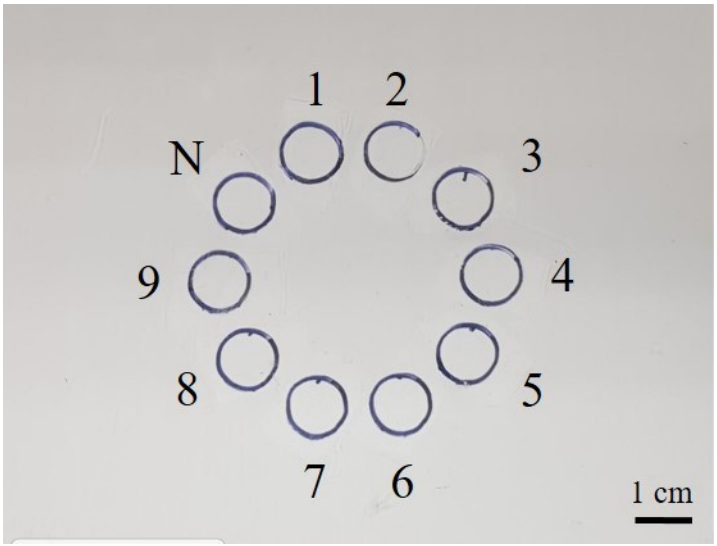


Figure S3. A circular glass array for spotting 6-mm tissue samples (either healthy or cancerous tissues could be applied). N=negative control region.

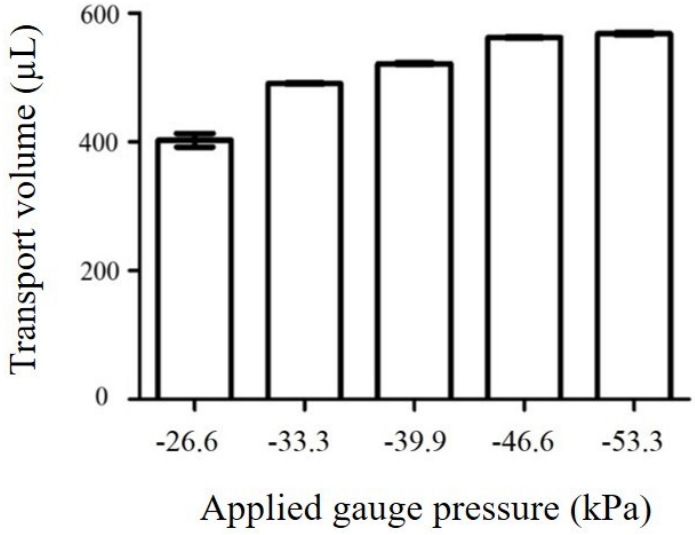
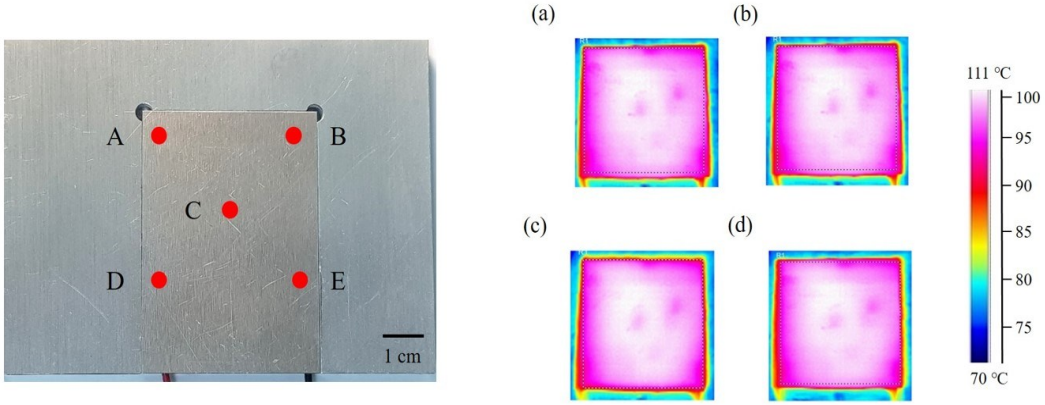


Figure S4. The relationship between the liquid transport volume and the applied (air) gauge pressure. Across 16 transport iterations, the variation in liquid volume transported remained low (error bars represent standard deviation).



(a) (b)

Figure S5. (a) Five points selected within the thermoelectric cooler for temperature measurements (external thermometer). (b) Temperature homogeneity on the thermoelectric (TE) cooler surface. During the 20-min heating process, the average temperature was ~108°C across the TE cooler surface. (a)-(d) represent the temperature distributions for 5, 10, 15, and 20 min of heating, respectively. The temperature distribution within the TE cooler surface within 20 min was found to be adequately uniform.

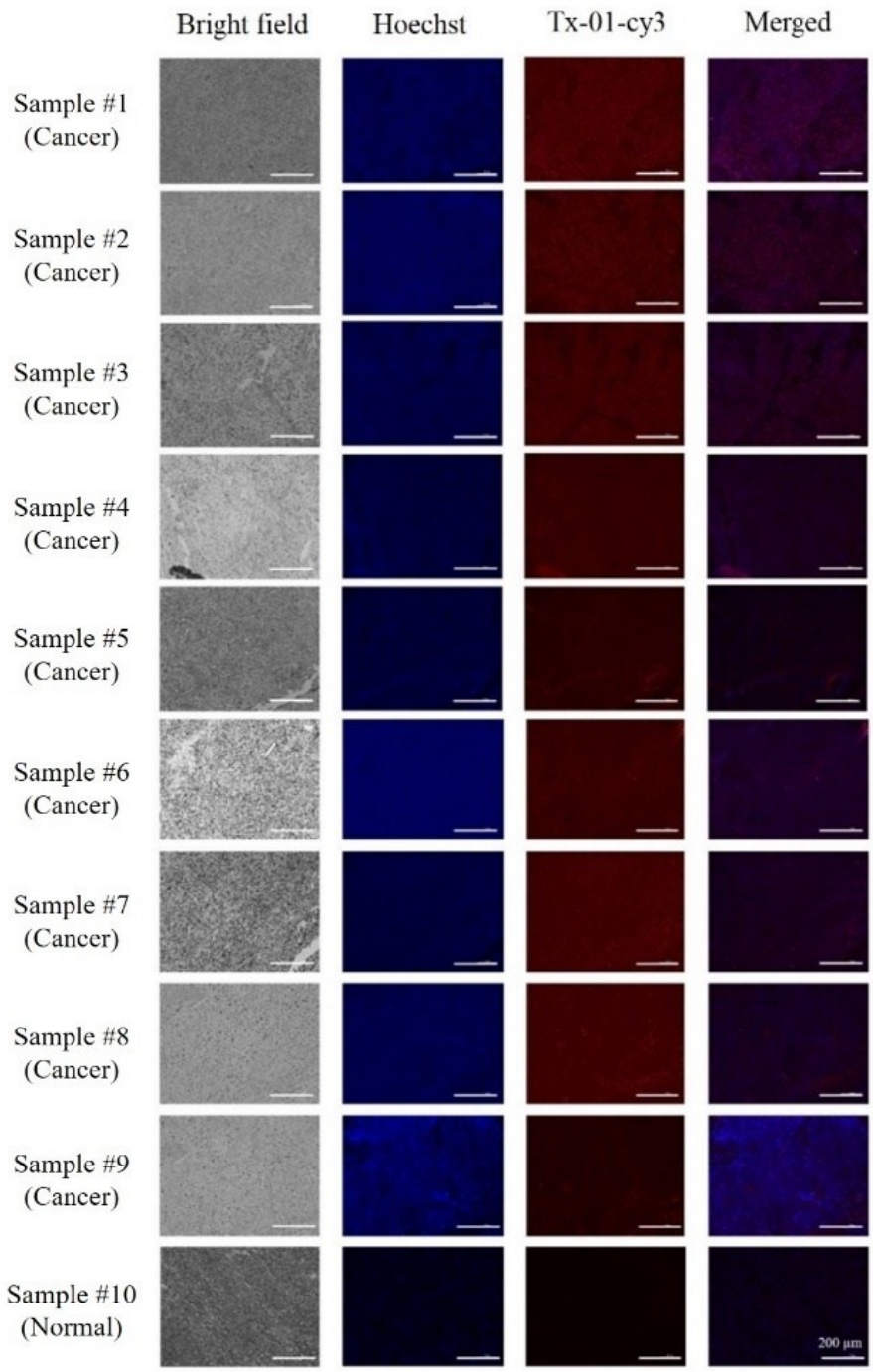


Figure S6. (a) Fluorescence results from on-chip, aptamer-based fluorescent staining; cancer cells could be distinguished from normal tissues within 80 min.