Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting Information

A Chip Assisted Immunomagnetic Separation System for Efficient Capture and *in-situ* Identification of Circulating Tumor Cells

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S.1 Reagents and instrument

Branched poly (ethylene imine) (PEI, MW 25 kDa and MW 750 kDa), tetraethyl orthosilicate (TEOS), (3-aminopropyl) triethoxysilane (APTES), polyvinylpyrrolidone (PVP-k30), N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), 4,6-diamidino-2-phenylindole (DAPI), bovine serum albumin (BSA), and anti-EpCAM monoclonal antibodies were purchased from Sigma-Aldrich. FITC labeled anti-Cytokeratin 19 (CK19) monoclonal antibodies and allophycocyanin (APC) labeled anti-CD45 monoclonal antibodies were got from Abcam. Indium tin oxide (ITO) glasses with the resistance of 10 Ω were purchased from LaiBao (LaiBao Hi-Tech Co.,Ltd., China). AZ50XT and AZ9260 photoresists were purchased from AZ Electronic Materials Corp.. PDMS and curing agent were obtained from GE Toshiba Silicones Co., Ltd. (Japan).

Breast cancer MCF-7 cells, liver cancer Hep G2 cells, and Jurkat T cells (human peripheral blood leukemia T cells), HL 60 cells (human promyelocytic leukemia cells) were purchased from China Type Culture Collection. Human blood samples were supplied by Hubei Cancer Hospital of Wuhan University and Hospital of Wuhan University. All the medias for cell culture were bought from Gibco Corp and ExCell Biology Inc..

Ultrapure water (18 MΩ·cm) was made by a Millipore Milli-Q system. Fluorescence images were recorded with a CCD camera (Nikon DS-Ri1) mounted on an inverted fluorescence microscope (Ti-U, Nikon, Japan) and emCCD (Andor DU-885K-C50-#VP-500) mounted on an inverted fluorescence microscope (Olympus IX70). UV-Vis absorption spectra were measured with an UV-Vis spectrophotometer (UV-2550, Shimadzu Corporation). The TEM images were obtained by a FEI Tecnai G2 20 TWIN electron microscope. The magnetic hysteresis loops were measured with a vibrating sample magnetometer (Lake Shore 7410 VSM). Dynamic light scattering (DLS) was performed on a Malvern Zetasizer Nano ZS instrument. All the masks used in the experiments were produced in Shanghai KaiSheng Electronic CO. Ltd., China.

S.2 Characterization of MNs were conjugated with anti-EpCAM antibody

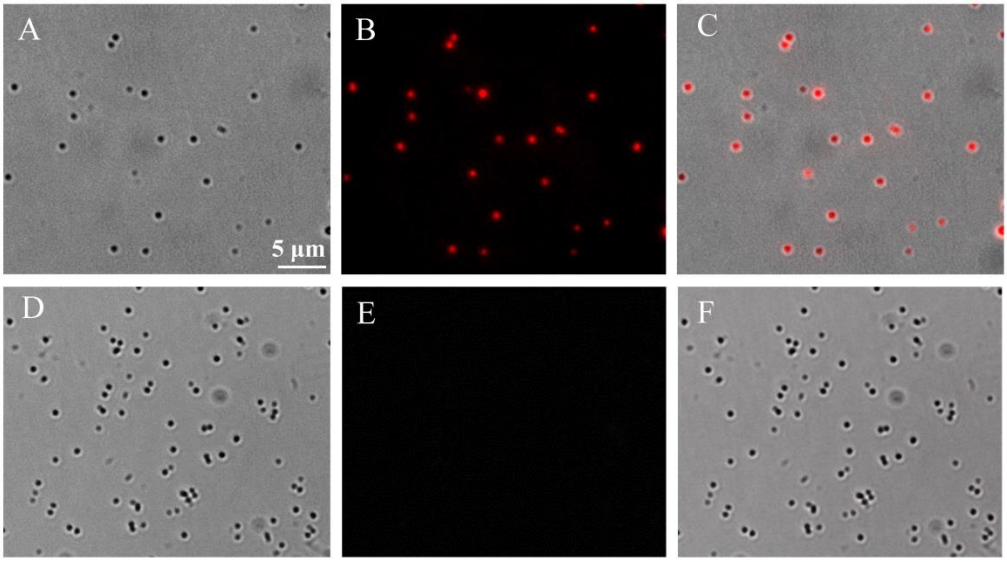

Fig. S1: Bright image, fluorescence image and merge of the two images of the IMNs (A, B, C) and MNs (D, E, F) after reacted with cy3-labeled rabbit anti-mouse IgG.

S.3 Characterization of the nickel patterns and IMN patterns

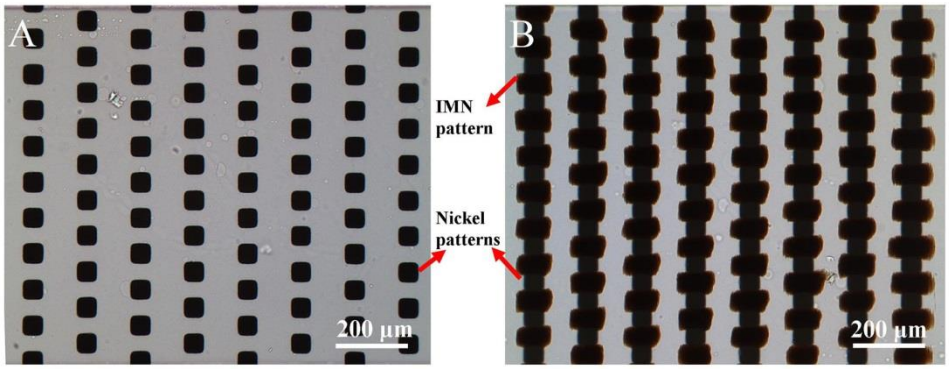

Fig. S2: Bright images of the nickel patterns (A) and the formed MNs patterns after introducing IMNs with flow rate 20 µL/min (B).

S.4 Characterization of the IMN patterns area

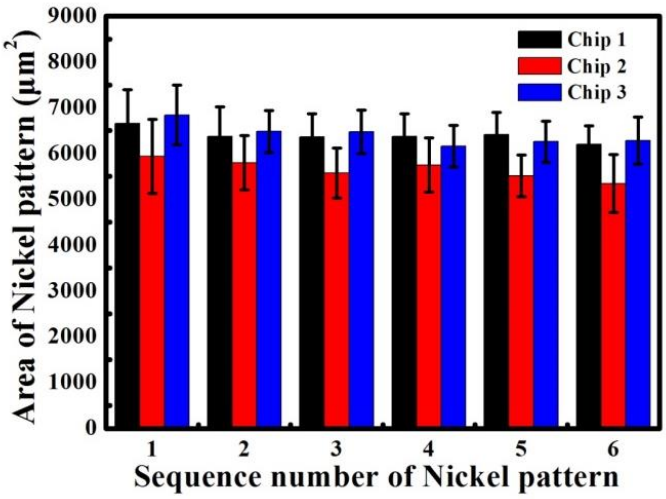

Fig. S3: Characterization of the IMN squares areas in 3 different chips. The IMN squares of different units in each chip had similar areas, showing the IMN patterns were very uniform.

S.5 Cells motion trajectories in structure-1 and in structure-2

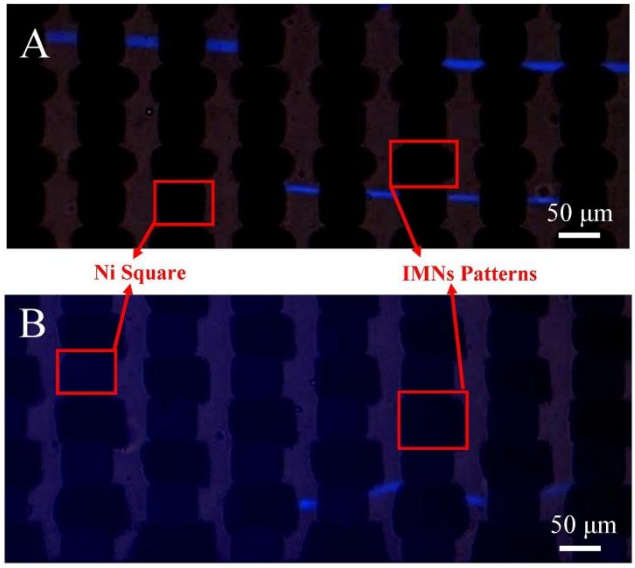

Fig. S4: Cells motion trajectories in structure-1 (A) and in structure-2 (B). The cell motion trajectories in structure-1 was straight line while in structure-2 was curving, revealing the arranged the nickel pattern in stagger can enhance the contact frequency between the IMN patterns and tumor cells. And the sizes of IMN patterns forming in these two structures were different, IMN patterns in structure-2 were much bigger than that in structure-1. The exposure time is 600 ms.

S.6 Cell distribution in the IMN patterns along X-axis direction

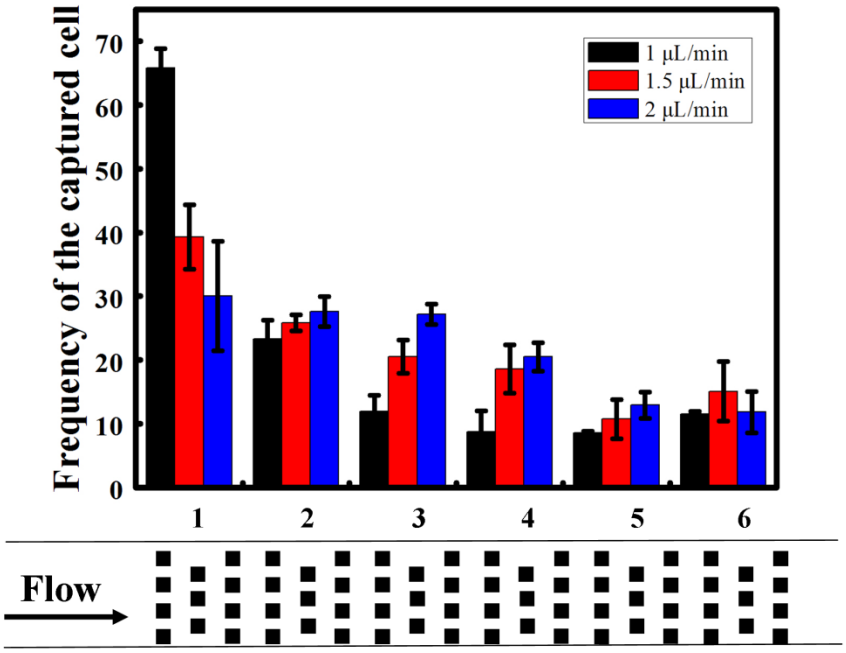

Fig. S5: Spatial distribution of captured MCF-7 cells along the IMN patterns at different flow rates. Error bars represent the standard deviations of triplicate experiments.

S.7 Enrichment of CTCs from patient peripheral blood samples

Table S1. Enumeration of CTCs in blood samples from patients with advanced metastatic cancer.

Sample No.	Cancer type	Gender	Volume processed/mL	CTCs
1	Lung	F	0.8	12
2	Lung	M	0.8	8
3	Lung	M	0.8	6
4	Lung	F	0.8	2
5	Lung	M	0.8	10
6	Lung	F	0.8	8
7	Gastric antrum	M	0.8	6
8	Gastric	F	0.8	9
9	Lymphatic metastasis	F	0.6	4
10	Liver	M	0.8	9
11	Health	Unknown	0.8	0
12	Health	Unknown	0.8	0
13	Health	Unknown	0.8	0