MICROFLUIDIC DEVICE FOR DNA AMPLIFICATION OF SINGLE CANCER CELLS ISOLATED FROM WHOLE BLOOD BY SELF-SEEDING MICRO-WELLS

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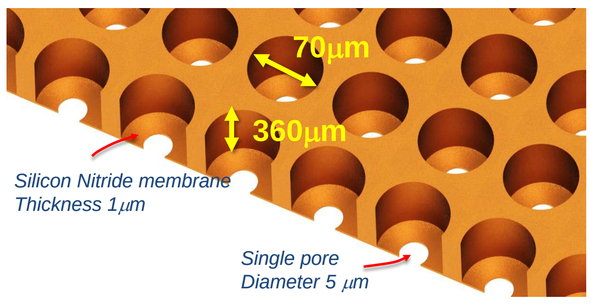
Introduction

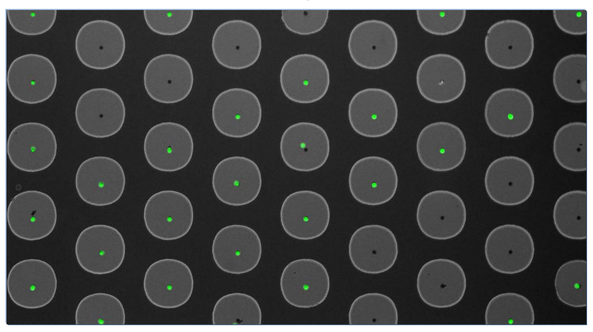
Genetic characterization of Circulating Tumor Cells (CTC) offers the opportunity for a “real time liquid biopsy” [1, 2]. Heterogeneity and rarity of CTC command the need for individual cell characterization. Following an enrichment procedure of CTC from blood, the identification, isolation and manipulation of single cells for further analysis without cell loss remains challenging. Self-seeding microwell plate can sort single cells into 6400 wells based on cell size and their identity verified by immunofluorescence staining [3]. Here, we developed a microfluidic device in which these single cells can be placed, lysed and their DNA amplified for further genetic analysis. Reagents were introduced by peristaltic pumping of micro-valves. On-chip lysis and amplification was performed in 8 parallel chambers.

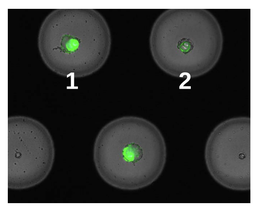
Self-seeding Microwell

Microwell Plate

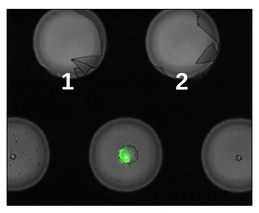


Fluorescence Image of Cells

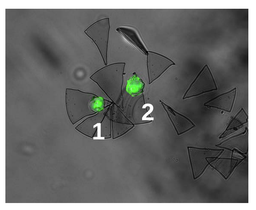




Select cells of interest



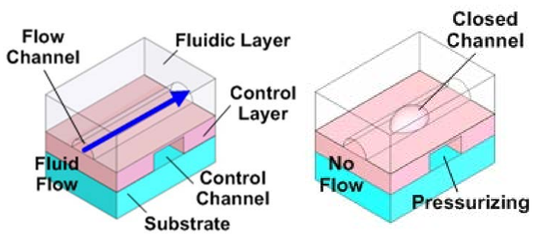
Punch out the bottom of microwell with cells



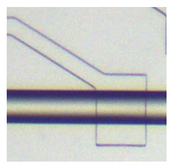
Punched cells

Open-well Microfluidic Device

Pneumatic Valve



< Open >



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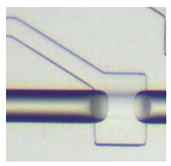
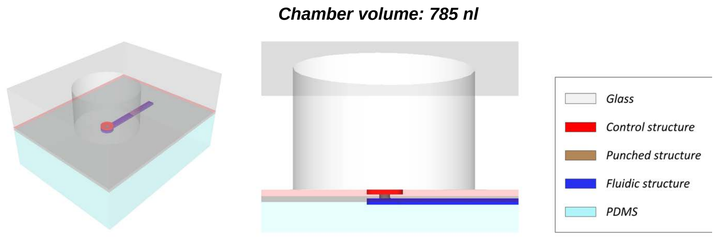
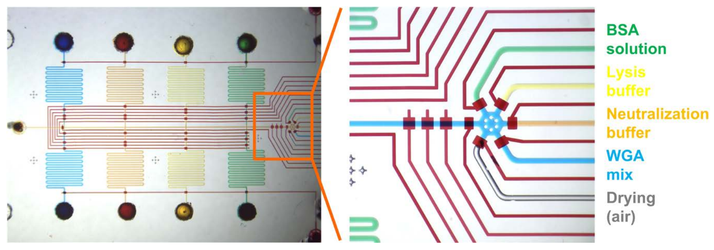


Device Design

Chamber volume: 785 nl





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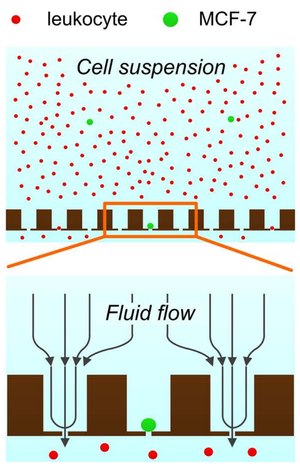
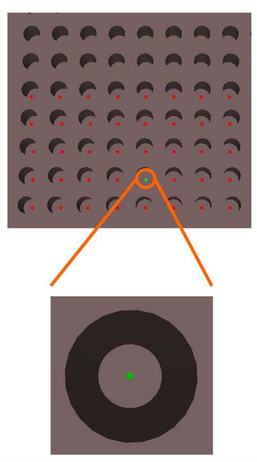
Single Cancer Cell Isolation and DNA amplification

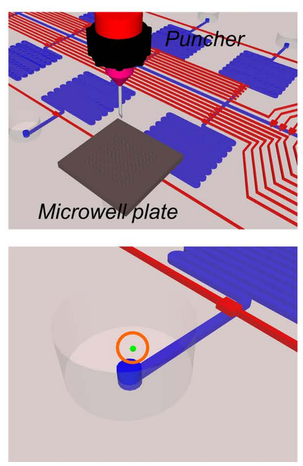
A. Filtration



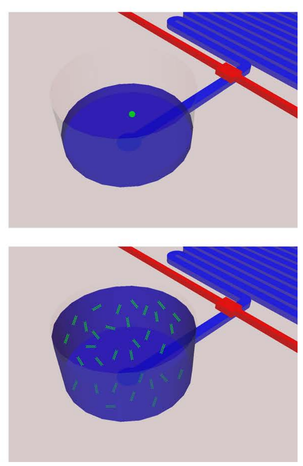
B. Detection

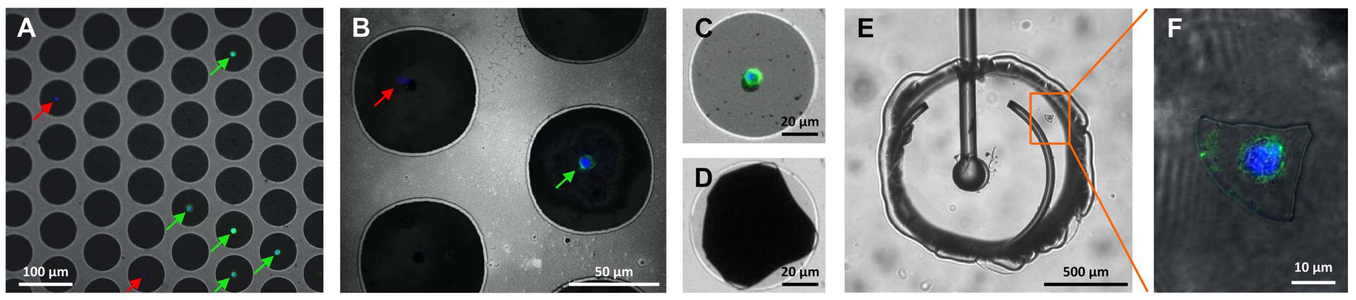


C. Punching



D. Lysis and amplification



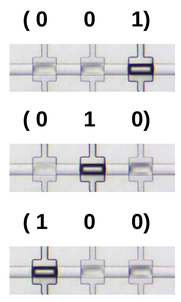


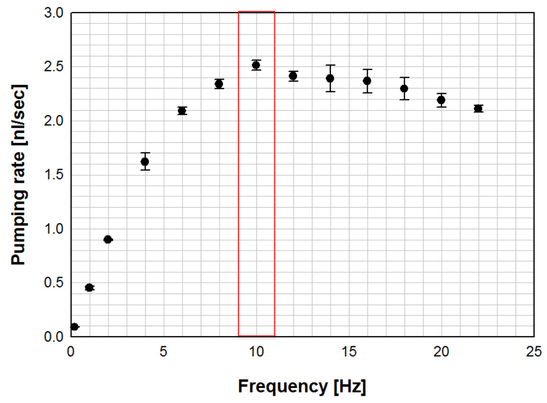
- Spiked 1000 of MCF-7 cells in 1ml of whole blood after leukocyte depletion.
- Identification of 37 % of MCF7 (Hoechst+/ EpCAM+) cells on the microwells.
- 10,000-fold DNA amplification in 1/100X reagent volume

Peristaltic Pumping

Pumping rate: 2.5 nl/sec (10 Hz)

Sequence





Validation of on-chip amplification

qPCR of 8 genes

Chambers		1	2	3	4	5	6	7	8	gDNA
# of cells		25	25	5	5	1	1	0	0	
Genes	**ERBB2** 17q12	■	■		■					■
	CCND1 11q13	■	■	■						■
	MyC 8q24	■	■	■	■		■			■
	PRMT2 21q22	■	■			■				■
	URB2 1q42	■	■							■
	P53 17p13	■	■	■			■			■
	TRAM1 8q13	■	■	■		■	■			■
	PAK1 11q13	■	■	■	■	■				■

■ Amplified ☐ Non-amplified

Conclusions

We developed a microfluidic device to enrich and isolate individual tumor cells from whole blood and amplify its DNA for further characterization.

References

[1] M. Cristofanilli, G. T. Budd, M. J. Ellis, A. Stopeck, J. Matera, M. C. Miller, J. M. Reuben, G. V. Doyle, W. J. Allard, L. W. Terstappen, D. F. Hayes, N Engl J Med, 351, 781-791,2004.
[2] A.M.C Barradas, L.W.M.M. Terstappen. Cancers 5(4), 1619-1642, 2013.
[3] Swennenhuis J.F.,Tibbe A.G.J.,Stevens M, Katika M, van Dalum J,Tong H.D. van Rijnd C.J.M., Terstappen LWWM, Lab Chip, 2015,15, 3039-3046

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