Single Cell in a WGA Cup : Open-well Whole Genome Amplification Device

Yoonsun Yang[1], Hoon Suk Rho[2], Michiel Stevens[3], Arjan GJ Tibbe[3], Han Gardeniers[2] and Leon WMM Terstappen[1]

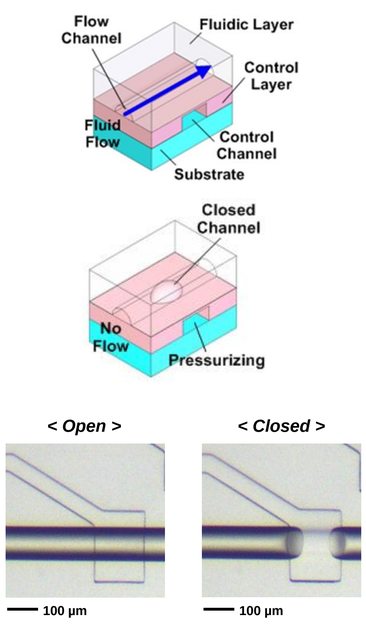
1. Medical Cell BioPhysics Group, MIRA Institute, University of Twente, The Netherlands
2. Mesoscale Chemical Systems Group, MESA+ Institute for Nanotechnology, University of Twente, The Netherlands
3. VyCAP B.V., The Netherlands

Introduction

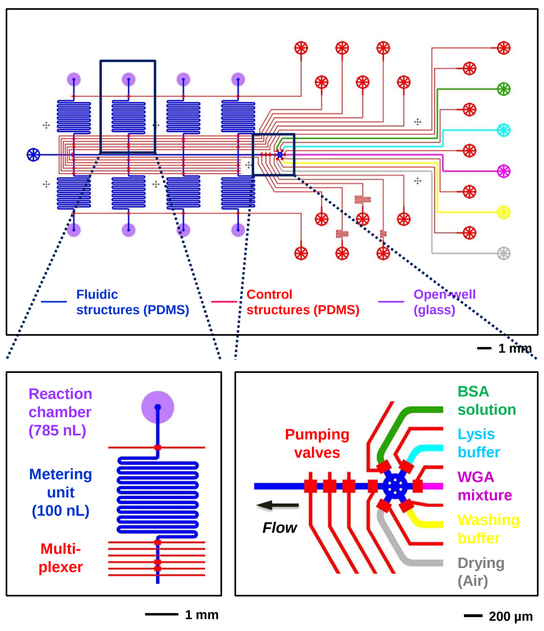
Genetic characterization of Circulating Tumor Cells (CTC) offers the opportunity for a "real time liquid biopsy" [1, 2]. Heterogeneity and rarity of CTC command the need for individual cell characterization. Following an enrichment procedure of CTC from blood, the identification, isolation and manipulation of single cells for further analysis without cell loss remains challenging. Here, we present a microfluidic device with open-well structures in which cells can be identified, isolated, lysed and the nucleic acids amplified following filtration. On-chip amplification will be a powerful tool to improve genetic analysis of single cells by making use of the smaller reagent volume, automation and parallel reactions of microfluidic devices [3].

Open-well Microfluidic Device

Pneumatic Valve

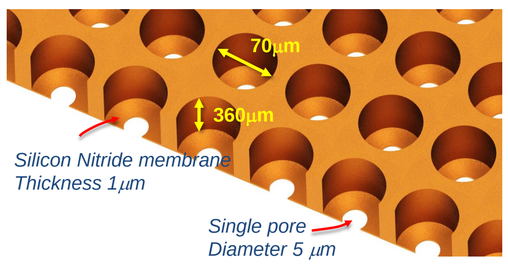


Device Design

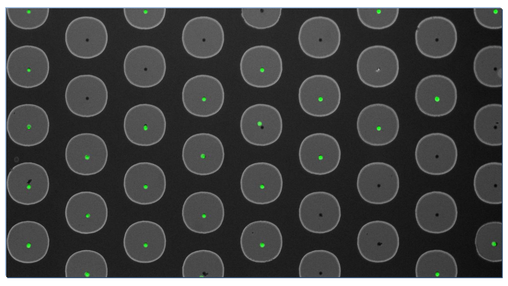


Self-sorting Microwell

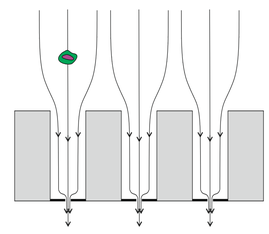
Microwell Plate Design



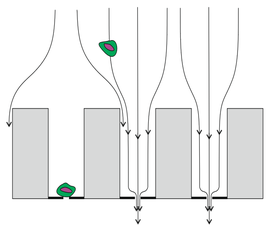
Cells in Microwells



Single Cell Seeding



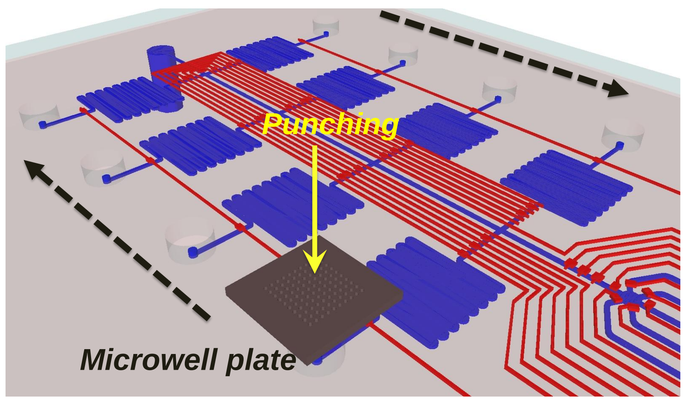
Fluid flow through open-pores at the bottom of microwells.



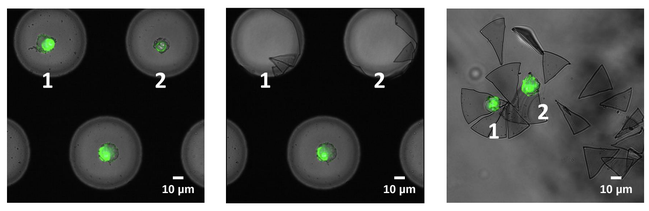
When single cell clogs the pore, fluid stop flowing. The next cell flows into the well without cells.

Single Cell Isolation

1. Filtrate thousands of cells through self-sorting microwell.
2. Scan self-sorting microwell plate under a fluorescence microscope.
3. Punch the bottom of microwells with cells into the reaction chamber.
4. Process isolated cell in the reaction chamber.

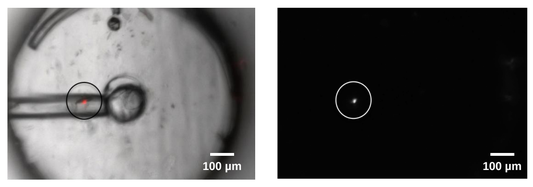


Images of punched cells



Select cells of interest | Punch out the bottom of microwell with cells | Punched cells

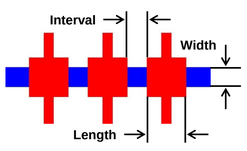
Isolated single cell in a reaction chamber



Peristaltic Pumping

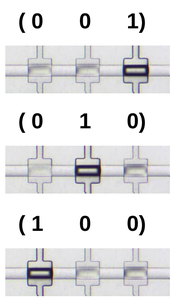
Loading reagents to reaction chambers

Design

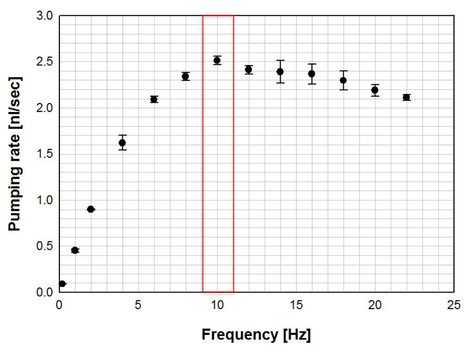


W (µm)	100
L (µm)	200
I (µm)	100

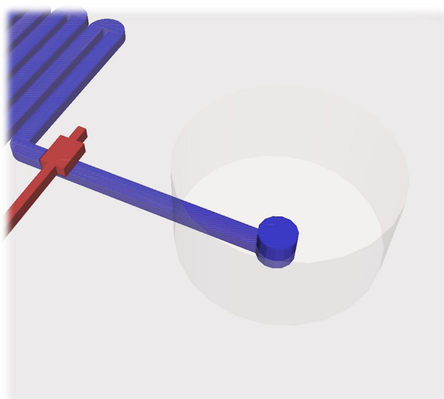
Sequence



Pumping rate: 2.5 nl/sec (10 Hz)



Whole Genome Amplification



Isolate single cells of interest

↓

Load proteinase K,~150 nl

Lysis ↓ 60°C, 1 h / 95 °C, 10 min

Load WGA mixture,~275 nl

Amplification ↓ 30 °C, 16 h

Collect amplified-DNA out

Conclusions

We developed a novel microfluidic device for whole genome amplification of single cell isolated by self-sorting microwell plate.

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] Yang Y, Swennenhuis JF, Rho HS, Le Gac S, Terstappen LWMM. PLoS ONE 9(9): e107958. doi:10.1371/journal.pone.0107958, 2014.

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