# Single Cell in a WGA Cup : Open-well whole genome amplification

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

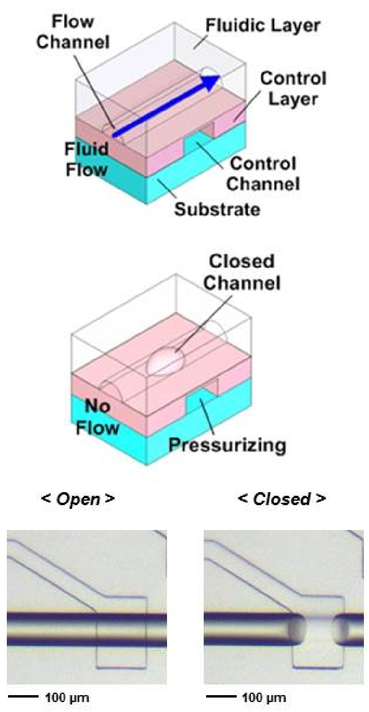
*1. Medical Cell BioPhysics Group, MIRA Institute, University of Twente, The Netherlands*
*2. Mesoscale Chemical Systems Group, MESA+ Institute for Nanotechnology, University of Twente, The Netherlands*
*3. VyCAP B.V., The Netherlands*

## *Introduction*

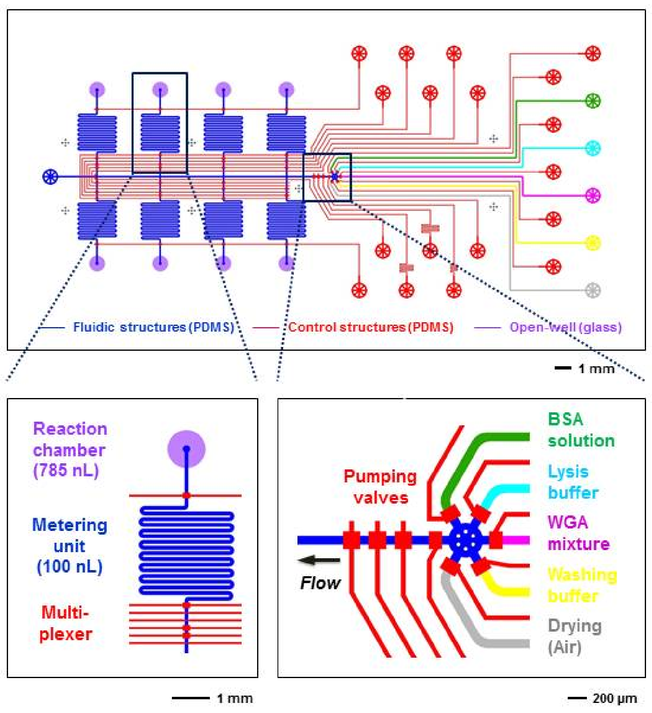
Genetic characterization of Circulating Tumor Cells (CTC) offers the opportunity for a "real time liquid biopsy" [1, 2]. Heterogeneity and rarity of CTC command the need for individual cell characterization. Following an enrichment procedure of CTC from blood, the identification, isolation and manipulation 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

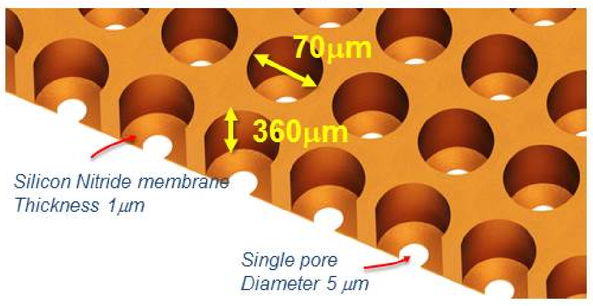



### *Device Design*

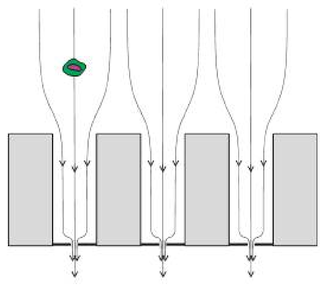



## *Self-sorting Microwell*

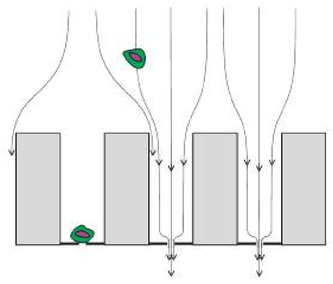
### Microwell Plate Design




### Single Cell Seeding

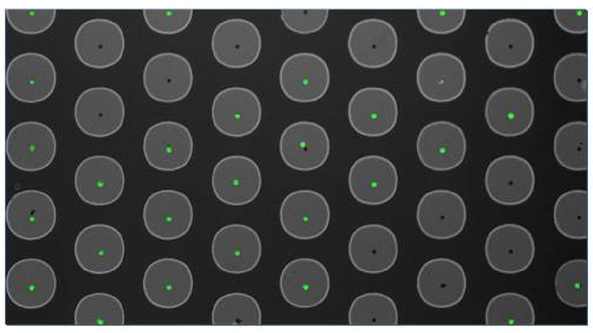


Fluid flow through open-pores at the bottom of microwells.



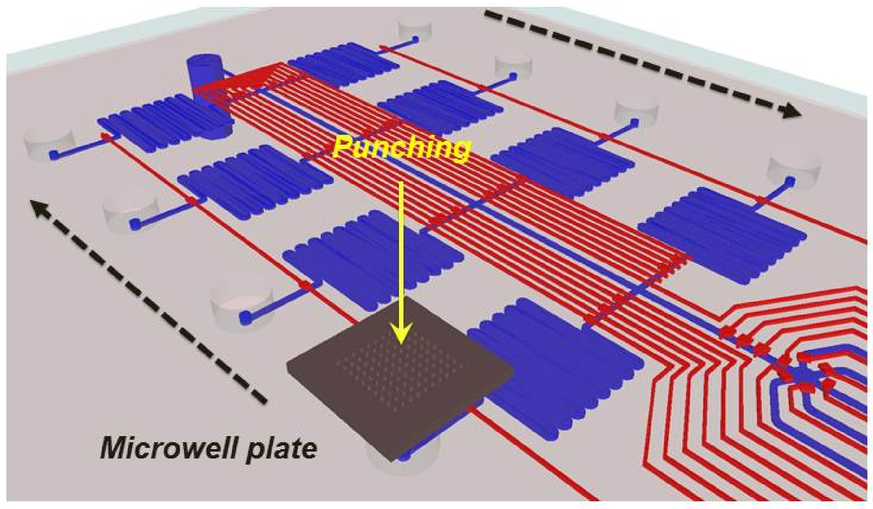
When single cell clogs the pore, fluid stops flowing. The next cell flow into the well without cells.

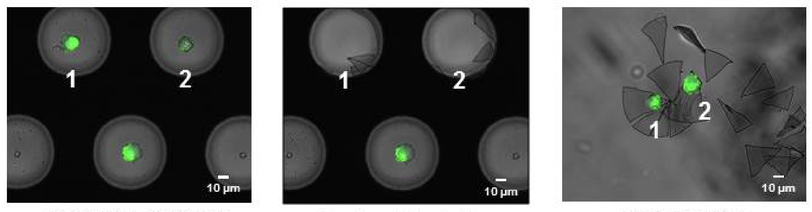
### Fluorescence Image of Cells in Microwells




## *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.



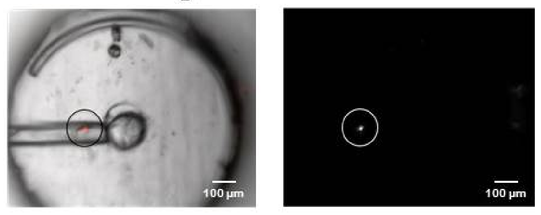




Select cells of interest

Punch out the bottom of microwell with cells

Punched cells

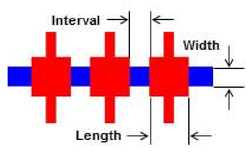
***Isolated single cell in a reaction chamber***




## *Peristaltic Pumping*

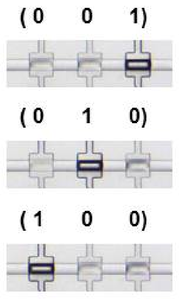
Loading reagents to reaction chambers using peristaltic pumping

***Design***

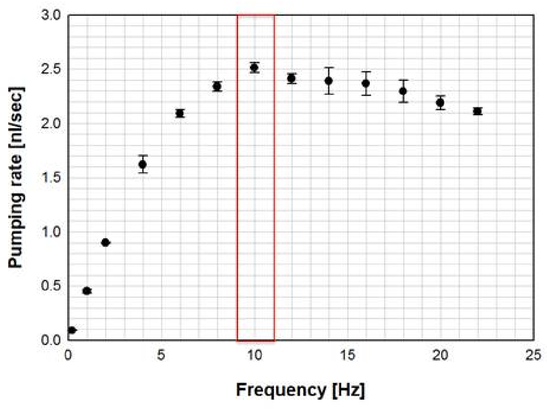



| | |
|---|---|
| *W (µm)* | *100* |
| *L (µm)* | *200* |
| *I (µm)* | *100* |

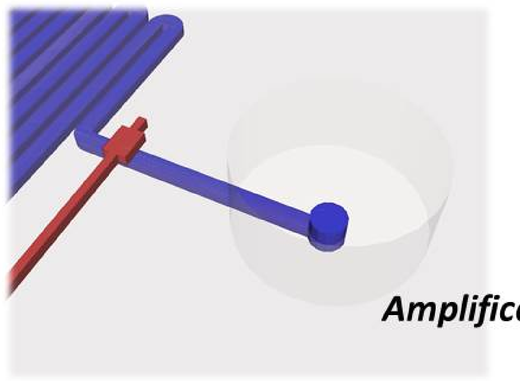
***Sequence***




***Pumping rate: 2.5 nl/sec (10 Hz)***




## *Whole Genome Amplification on a Chip*



***Amplification***

***Lysis***

*Isolate single cells of interest*

↓

*Load proteinase K,~150 nl*

↓ 60°C, 1 h / 95 °C, 10 min

*Load WGA mixture,~275 nl*

↓ 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.

UNIVERSITY OF TWENTE. MIRA INSTITUTE FOR BIOMEDICAL TECHNOLOGY AND TECHNICAL MEDICINE MESA+ INSTITUTE FOR NANOTECHNOLOGY MCBP VyCAP nanonextnl innovating with micro and nanotechnology