# HIGH-THROUGHPUT GENE EXPRESSION ANALYSIS OF SINGLE CELLS USING DIGITAL MICROFLUIDICS

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## ABSTRACT

As a step towards automation of single cell gene expression analysis, we propose here a novel system able to perform single cell encapsulation in nanoliter (nL) droplets and rapid distribution in a microtiter plate . The process allows high-throughput compartmentalization of hundreds individual cells in a microfluidic polydimethylsiloxane (PDMS) chip. Droplets containing reagents and individual cells are then distributed in a microtiter plate where lysis, efficient reverse transcription (RT) of the RNA content of each cell and amplification are performed.

**KEYWORDS:** Droplets generation, Droplets distribution, Single cells, encapsulation, Reverse transcription

## INTRODUCTION

Gene expression analysis in single cells constitutes a major issue in understanding the complexity of living organisms but remains a challenge due to the small amount of material present in individual cells.

It was previously shown that RT performed in a nanoliter PDMS rotary mixer is more efficient as compared to conventional RT performed in microliter volumes (µL) [1]. However whole-amplification of the produced cDNAs was shown to require µL volumes. In addition only 2-3 cells per day could be analyzed with this rotary mixer. In order to increase the throughput, digital microfluidics integrating one-step high-throughput single cell trapping, lysis and RT-PCR in droplets was applied [2]. A microfluidic system was built for encapsulating cells in nL droplets together with RT reagents and for recovering the droplets in microtiter plates held on a translation-stage.

## EXPERIMENTAL

A PDMS chip was designed with two inputs for aqueous phases and one input for mineral oil, a flow focusing junction and a straight output. A schematic view of the set-up is shown in figure 1. RT reagents and cells are injected in the system upstream from the junction. Microfluidic pneumatic valves [3] and a pressure controller allow adjustment of relative concentration, droplet volume and droplet formation time. Droplets are recovered in a microtiter plate at the output of the microfluidic chip.

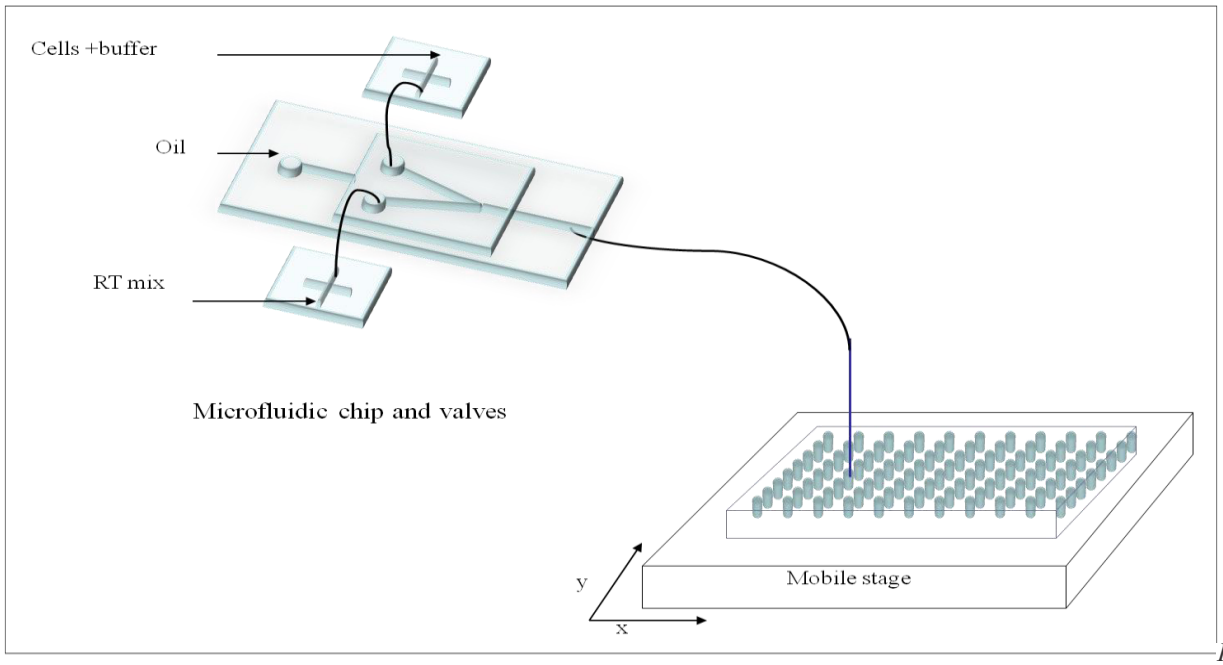


*Figure 1 : Experimental setup for droplet generation and distribution.*
*Droplets containing reagents are generated in a microfluidic PDMS chip and then distributed in a microtiter plate where lysis, RT and then TS-PCR are performed*

The aqueous phases are in contact 100 µm upstream the cross junction so that the lysis and RT reaction will not start before isolation of cells in individual compartments. Aqueous droplets are generated at the flow focusing junction and carried by the oil, as shown on figure 2

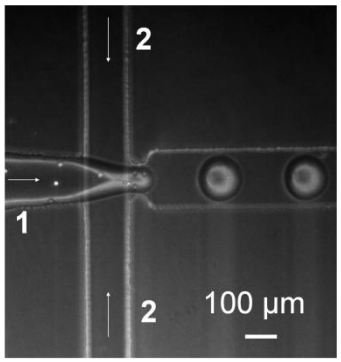


*Figure 2 : Droplet formation at the flow focusing junction.*
*Inlet 1 : Aqueous phase with cells and RT reaction buffer. Inlet 2 : oil.*
*At the cross junction, the oil flow is pinching the interface of the aqueous phase breaking it up into droplets, further flowed downstream. Droplets are carried to a microtiter plate were the RT reaction is performed.*

The output of the microfluidic system is connected to a tubing above a microtitration plate. Droplets generated in the system were recovered in wells of a microtitration plate as shown in figure 3. The plate is filled with oil allowing aqueous droplets to sink in wells and avoiding air contact and evaporation. Translation of the stage allows to get one drop per well.

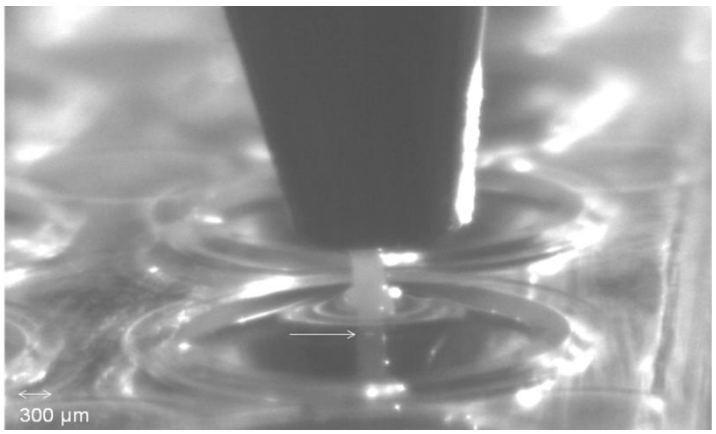


*Figure 3 : Droplet distribution. Droplets formed in the chip are distributed in a microtiter plate. The arrow indicates the position of the droplet in the tubing.*

RT reaction was performed in the nL droplet-reactors at 42°C. cDNAs were further amplified using template switching-polymerase chain reaction (TS-PCR) [4] in µL volumes. Labeled amplified cDNAs will finally be hybridized to DNA microarrays.

## RESULTS AND DISCUSSION

In order to test RT efficiency, RT was initially performed in nL droplets stored in a PDMS microfluidic chamber during the RT reaction as reported at µTAS 2009 [5]. All droplets were collected, diluted and the DNA content amplified using TS-PCR. On the electrophoretic gel shown in figure 4, the amplification of mouse universal RNAs by RT-TS-PCR was shown to be more efficient when RT was performed in droplets as compared to RT performed in tube. Amplification products were detected as a smear on the agarose gel with as little as 3.5 pg of starting RNA, corresponding to the quantity of RNA present in a single cell. We thus demonstrated the validity of a digital microfluidic approach for single cell gene expression profiling.

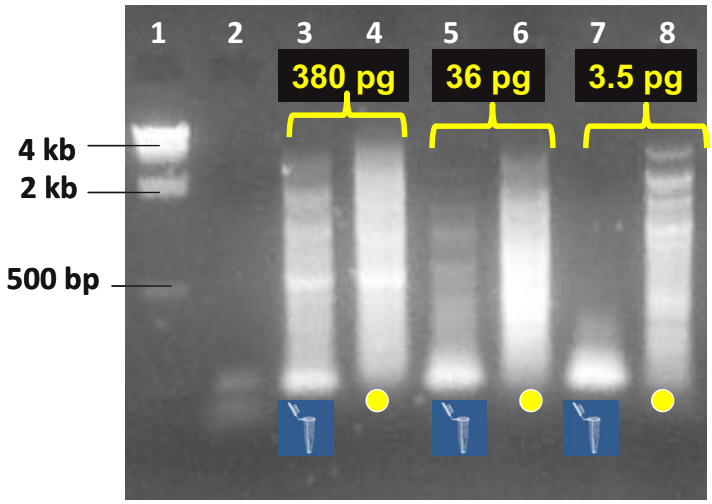


***Figure 4. Electrophoretic gel after TS-PCR.***
***RT was performed in droplets (nL) or in tube µL. Amplification was performed in tube.***
***1: DNA ladder***
***2: TS-PCR negative control***
***3 & 5 & 7: RT in tube 10µL (380pg RNA)***
***4 & 6 & 8: RT in 4nL droplets***

## CONCLUSION

With our new set-up, we will be able to isolate individual cells in droplets reactors and perform the initial steps (i.e. lysis and RT) in nL volumes in order to gain higher efficiency. Each droplet will be recovered in a separate well of a microtiter plate where TS-PCR amplification will be performed. We expect encapsulation of the cells in droplets and distribution in the microtiter plate to take a few min only. From a biological sample made of thousands cells, we will be able to obtain, for the first time, gene expression profiles of hundreds individual cells and look for discriminant markers.

## ACKNOWLEDGEMENTS

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