Development of segmented-flow microfluidic operations for single-cell nucleic acid analysis

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Introduction

The importance of single cell analysis is rapidly growing especially in the fields of biological and medical research. A cost- and time-efficient technology for handling and analyzing individual cells instead of averaging whole culture populations is extremely attractive for applications like drug development, pathological screening, cancer development and many others. Segmented-flow microfluidics is thought to be highly suitable for this purpose, considering the ability to handle a series of minute reaction compartments at moderate-to-high throughputs.

Our on-going development of segmented-flow microfluidic devices aims for the realization of an integrated, automated single-cell nucleic acid analysis system with complete sample preparation capabilities. Although some of the required unit operations such as droplet mixing and thermocycling are already fairly established in this field, key procedures such as accurate single-cell encapsulation and nucleic acid purification are still missing.

Methods

Most conventional methods for single-cell encapsulation into droplets show stochastic behaviour. This leads to the generation of droplets with multiple or no encapsulated cells, and therefore many false positives or negatives are detected within the series of droplets. We are currently developing fluidic methods to allow only single-cell-laden droplets to be transported through the microchannel structure.

Lysis of cells combined with the purification of nucleic acids is also an essential process for nucleic acid analysis. As extraction method, we have chosen solid-phase extraction using magnetic microparticles. Particle washing is an unexploited major topic in segmented-flow microfluidics, and our currently investigated approach is to transfer magnetic particles between droplets of different liquids with minimal reagent carryover.

Conclusion

Successful implementation of the above-mentioned microfluidic operations would be a significant step towards the construction of a sample-in results-out segmented-flow system for single cell analysis. The early results on the microfluidic development and the prospects for integration with the already established segmented-flow PCR amplification technology will be presented.