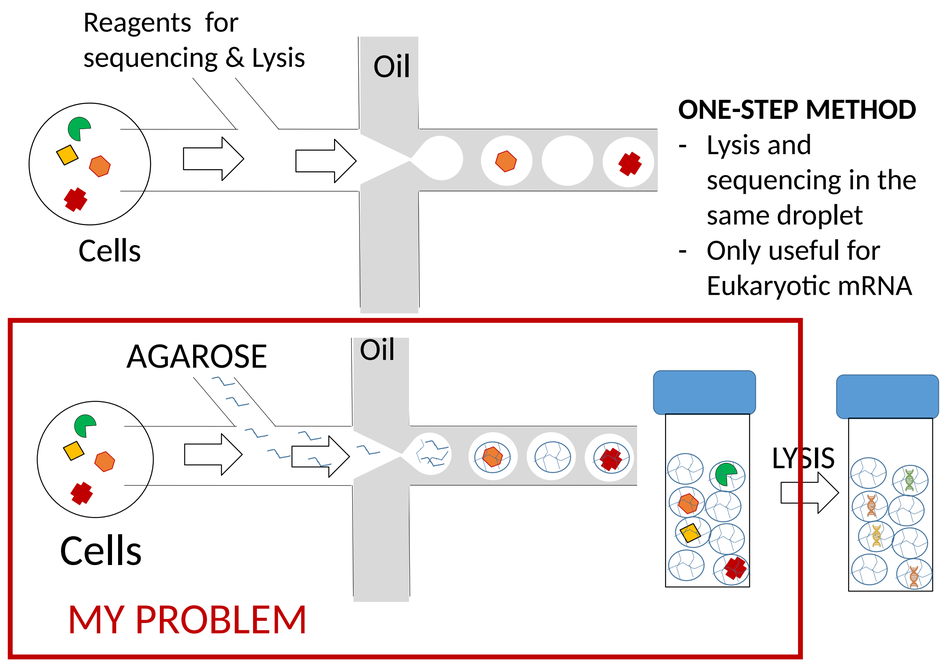
DESIGN AND DEVELOPMENT OF A MICROFLUIDIC PLATFORM OF ENCAPSULATION FOR THE ANALYSIS OF INDIVIDUAL GENOMIC CELLS

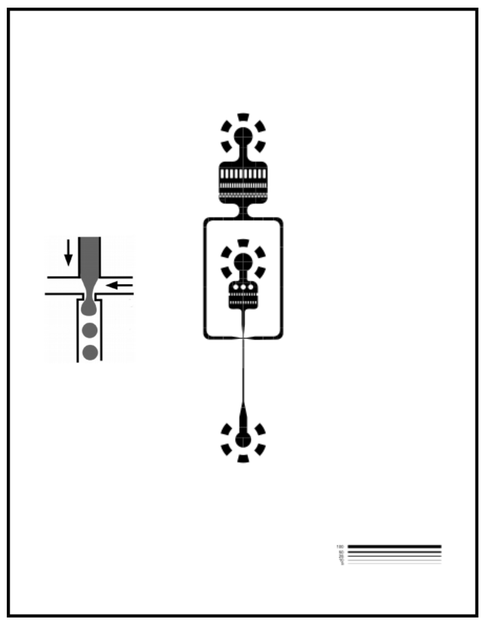
Javier Gutiérrez Solórzano, Raúl Fernández López
University of Cantabria, University of Basque Country
Institute of Biomedicine and Biotechnology of Cantabria

Single-cell sequencing (S-cs) allows the determination of the RNA/DNA sequence of individual cells within a population. Droplet-based microfluidics is particularly suited for S-cs, because its ability to encapsulate thousands of cells in individual droplets. However, current methods are based on a one-step encapsulation procedure in which cell lysis and sequencing reagents act sequentially in the same droplet. This limits the intensity of the cell lysis achievable, and as a consequence droplet based S-cs is mostly limited to RNAseq in eukaryotic cells. In order to expand the power of S-cs to prokaryotic and eukaryotic genomics, here we implemented the first phase of a two-step method suited for DNA sequencing in single cells. This first phase consists in a microfluidic system able to encapsulate cells into an agarose encaging. These solid agarose cages can then be lysed together, washed and re-encapsulated for DNA sequencing. To achieve the first part of this two-step procedure, we built a microfluidic chip, a tailored microscope stage and a calefactory circuit. Our systems allowed the controllable encapsulation of test cells. We optimized the process, by calculating and experimentally validating the cell concentration required for optimal droplet occupancy. These results can now be used to lyse and re-encapsulate these agarose beads, and achieve genomic S-cs in prokaryotic cells.

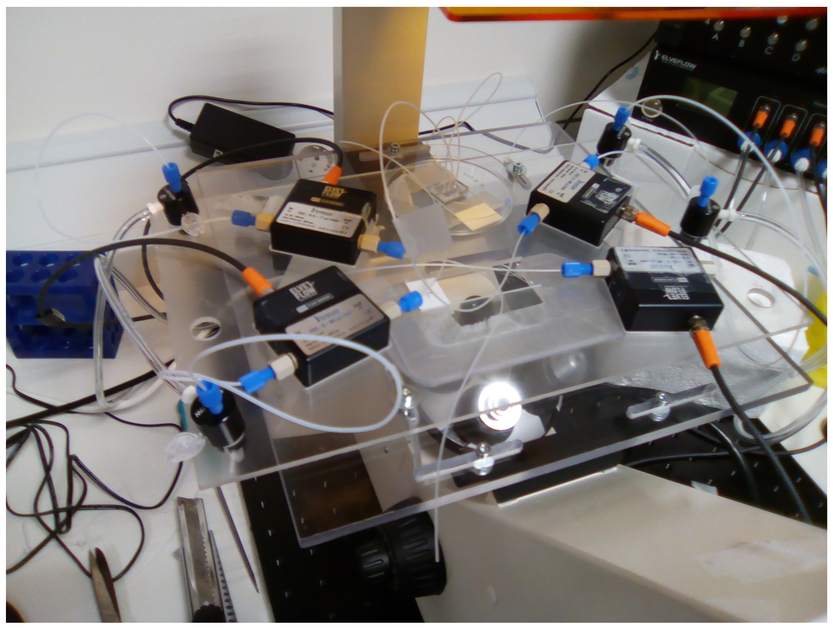
Introduction



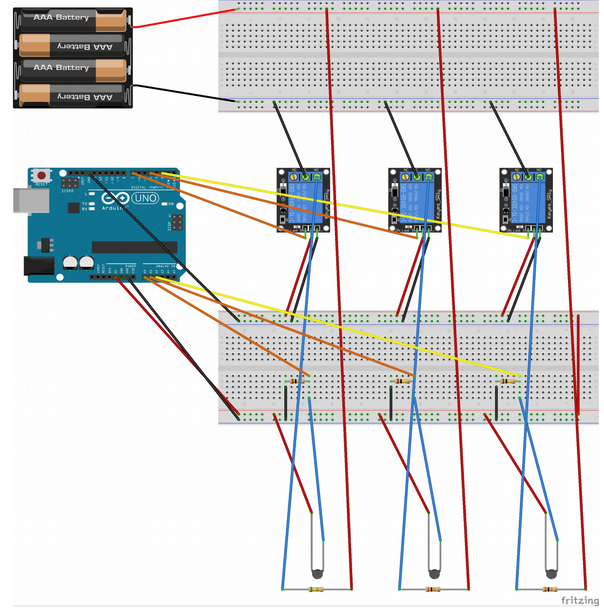
Methods and device



3-hole chip.



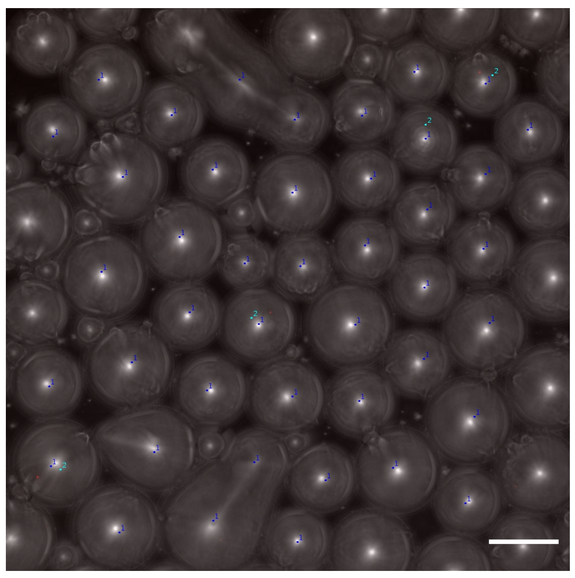
Microscope stage.



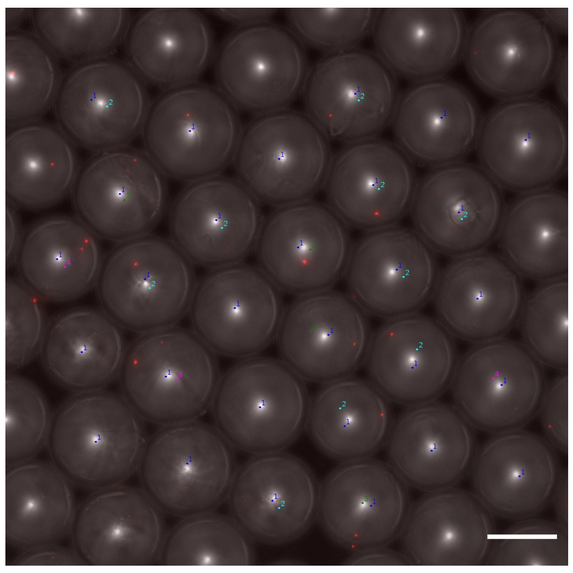
Electrical scheme of the system that allows to maintain the agarose above its melting point.

Results

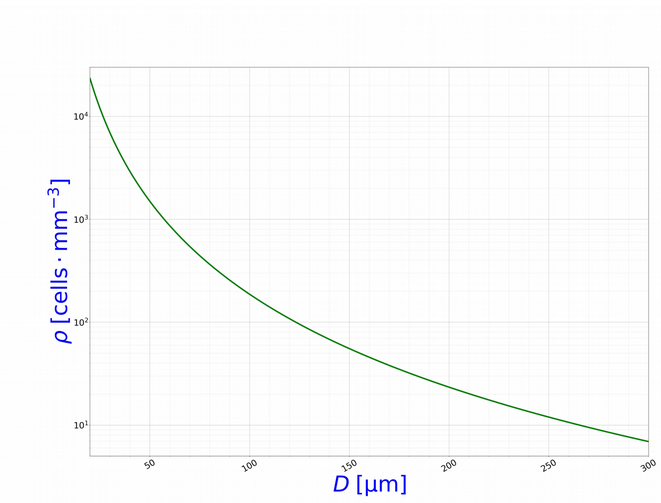
The final flows to make the encapsulates were 2.25 µl min^{-1} for the dispersed phase (agarose) and 6 µl min^{-1} for the continuous phase.



Expected: λ=0.0544 , 5% single >1% multiple
Obtained: λ=0.129, 11% single, 2% multiple



Expected: λ=0.976 , 37% single 26% multiple
Obtained: λ=0.961, 29% single, 31% multiple



Optimum density-diameter ratio to get a properly encapsulation frequency.

Conclusions

- We built and validated a microfluidic system able of controllable encapsulation of prokaryotic cells.
- Our calefactory system allows the controlled cooling of the agarose, without disturbing the fluxes.
- Our theoretical model and experiments showed that 1 cell per 10 volumes of encapsulated media allows optimal occupancy.

References

- Lan, F., Demaree, B., Ahmed, N., & Abate, A. R. (2017). Single-cell genome sequencing at ultra-high-throughput with microfluidic droplet barcoding. Nat Biotechnol, 35(7), 640-646.
- Joensson, H. N., & Andersson Svahn, H. (2013). ChemInform Abstract: Droplet Microfluidics - A Tool for Single-Cell Analysis. ChemInform , 44(21).
- Hirose, T., Hoshino, Y., Yoon, D., Nakahara, A., Mori, T., Sekiguchi, T., Takeyama, H., & Shoji, S. (2012). UNIFORM AND HIGH THROUGHPUT AGAROSE GEL MICRO DROPLET GENERATION DEVI CE F OR SINGLE CELL ANALYSIS. Retrieved June 16, 2019, from https://www.rsc.org/images/loc/2012/pdf/M.3.75.pdf