

## Summary

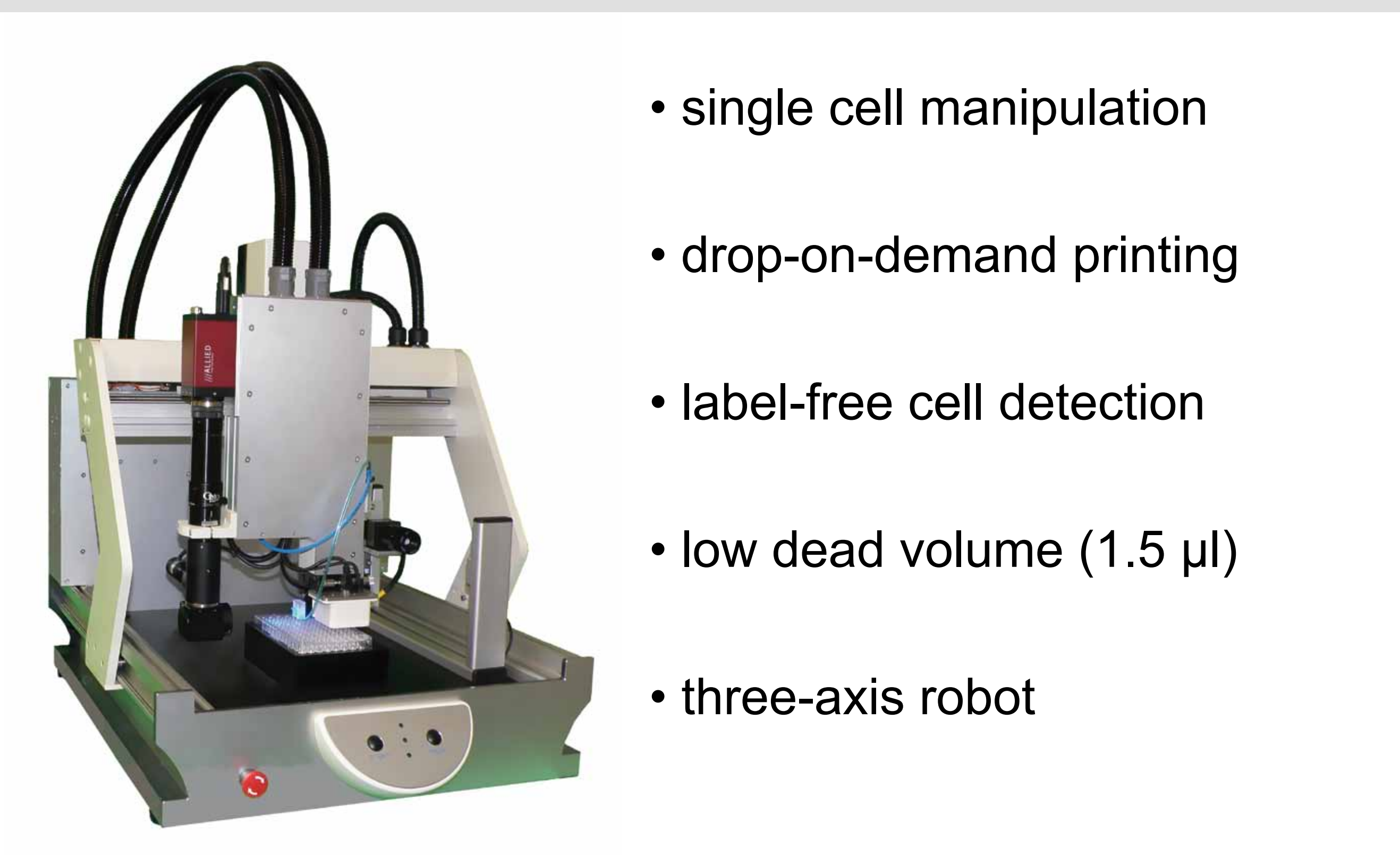
We present the application of the Single Cell Manipulator (SCM, [1]) for a proof-of-concept study about real-time single cell PCR in a commercial off-the-shelf thermocycler. The experiment features:

- Optical cell detection in dispenser
- Printing of single cells into PCR tubes
- Real-time PCR of single cells

Single cell analysis, including single cell PCR, is an emerging method of life sciences [2, 3]. Applications of the SCM technology include generation of clonal cells lines, heterogeneity studies, cancer and stem cell research.

## Single Cell Manipulator (SCM)

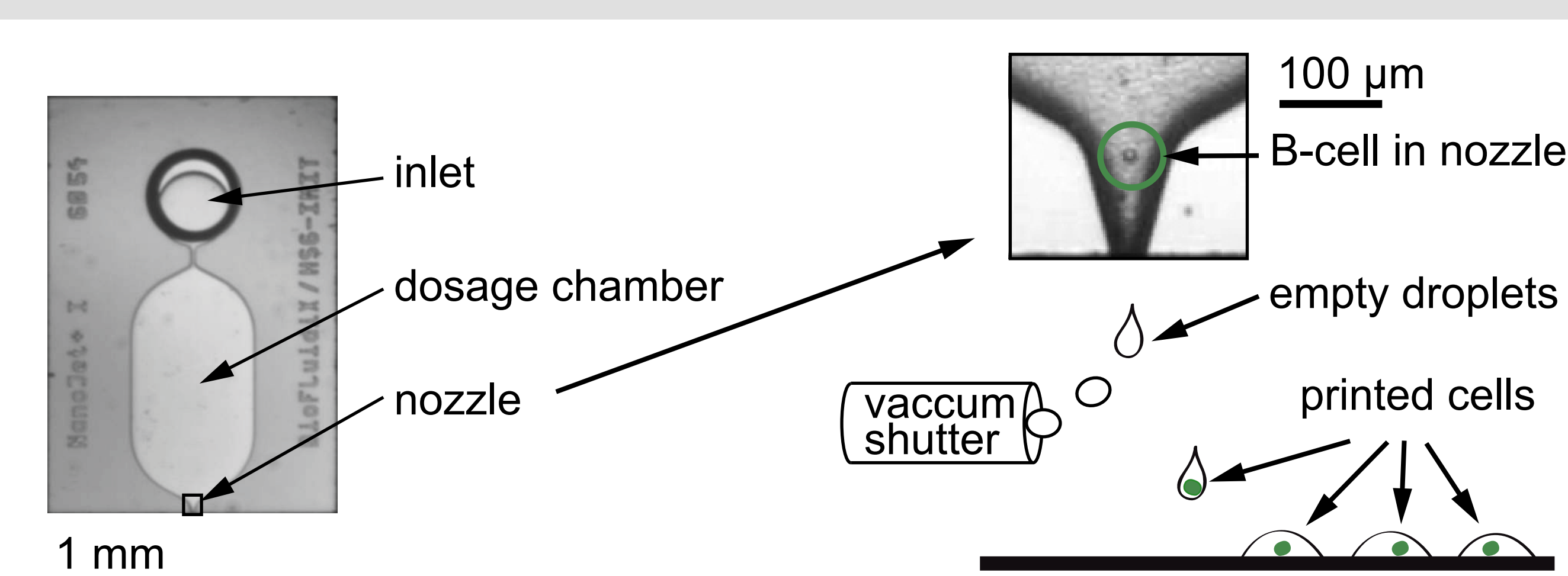
The SCM instrument (Fig. 1 & 2) consists of a three-axis robot, optics and a transparent dispenser chip. When a single cell is optically detected in the nozzle, it can be dispensed on demand encapsulated in a free-flying droplets (100 pl). Cell detection can be performed by the operator or automatically by a detection algorithm which enables higher through-put.



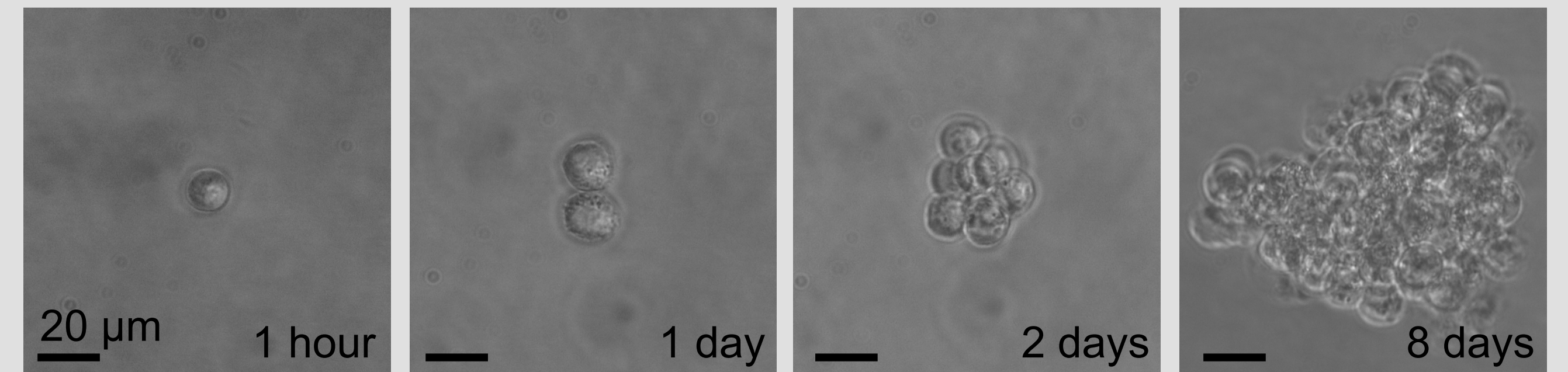
**Figure 1:** Single Cell Manipulator (SCM). Non-contact printer for the separation and positioning of single cells.

## Single Cell Printing

Manual B cell dispensing into a micro well plate revealed that 20 out of 23 droplets contained a single cells, one droplet contained two cells and two droplets were empty.



**Figure 2:** NanoJet cell dispenser chip fabricated from silicon and glass and single cell printing.

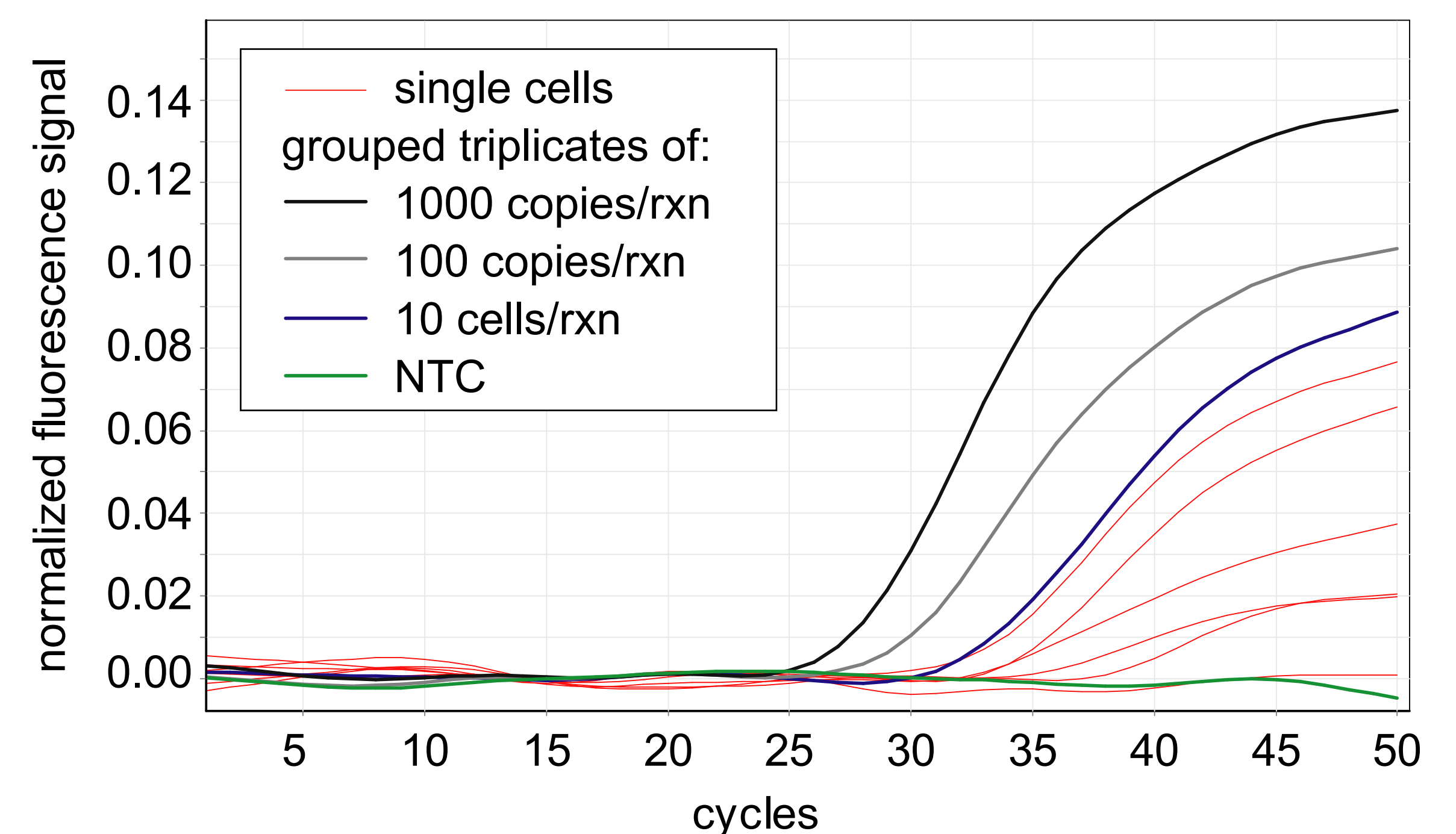


**Figure 3:** Single B cell printed into a well and cultured for 8 days.

Fig. 3 shows a single B cell printed into a micro well. It has been cultured over 8 days and proliferation has been observed. From 20 printed single cells 11 divided.

## Single Cell PCR

First experiments for single cell PCR with the SCM and a Rotor-Gene 2000 thermocycler (Corbett Research, now Qiagen) have been evaluated with B cells and a primer system against  $\beta$ -actin. B cells are suspended in PBS ( $10^5$  cells/ml) and printed manually into PCR tubes, pre-filled with 20 µl of PCR mix. Amplification signals from 20 of 76 single cell samples could be detected. Fig. 4 shows results from an exemplary data.



**Figure 4:** Result of a single cell PCR experiment. Positive signals were measured in 5 out 6 single cells samples.

## Conclusion

Non-contact printing of single cells is a versatile method to position cells individually on any uncovered substrate. Single cell PCR was shown as exemplary application.

## Acknowledgements

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## References

- [1] "Inkjet-like printing of single-cells", A. Yusof et al., Lab on a Chip, 11, 2447 (2011)
- [2] "Single-cell analysis: The deepest differences", C. Schubert, Nature, 480, 133 (2011)
- [3] "Microfluidic Digital PCR Enables Multigene Analysis of Individual Environmental Bacteria", A. Ottesen et al., Science, 314, 1464 (2006)