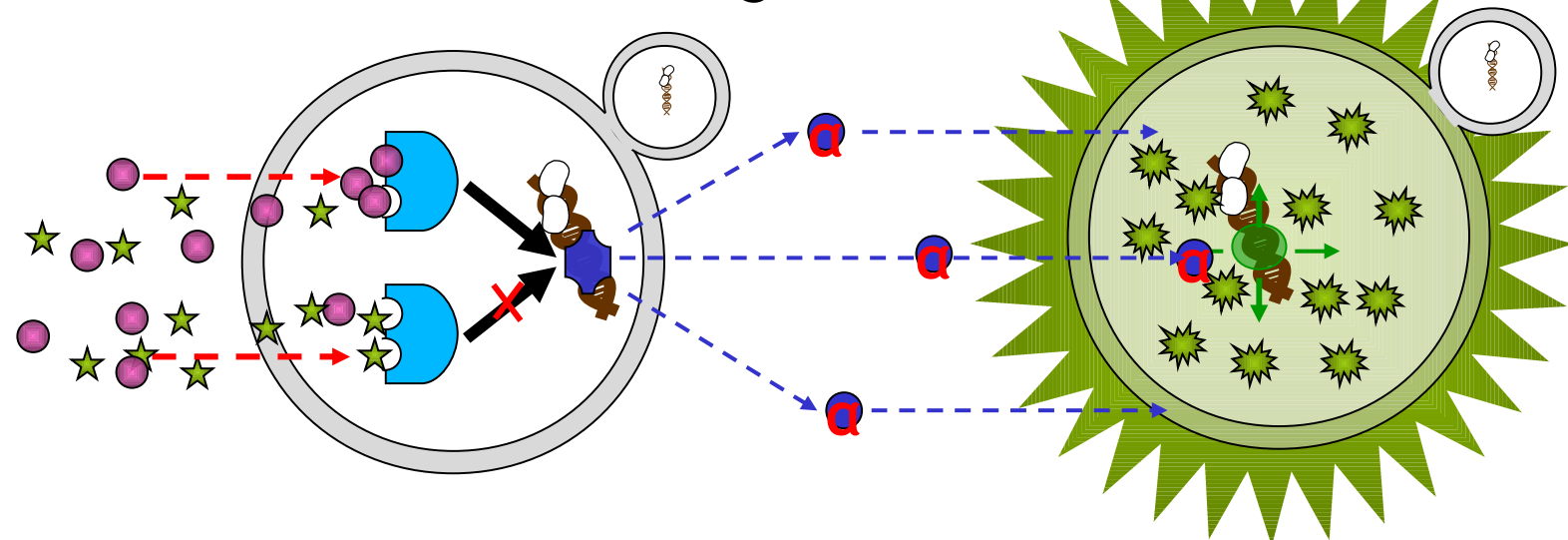


Motivation

- Need for safe and quick sensors and bioindicators for environmental and medicinal use
- Advances in eGFP biosensors and molecular engineering of yeast
- Limitations in biosensor design:
 - Problem to switch off and on in response to stimulus
- Options:
 - Development of sensor-actor biosystem
 - Study of ecological chemistry by yeast cell communication through α -factor

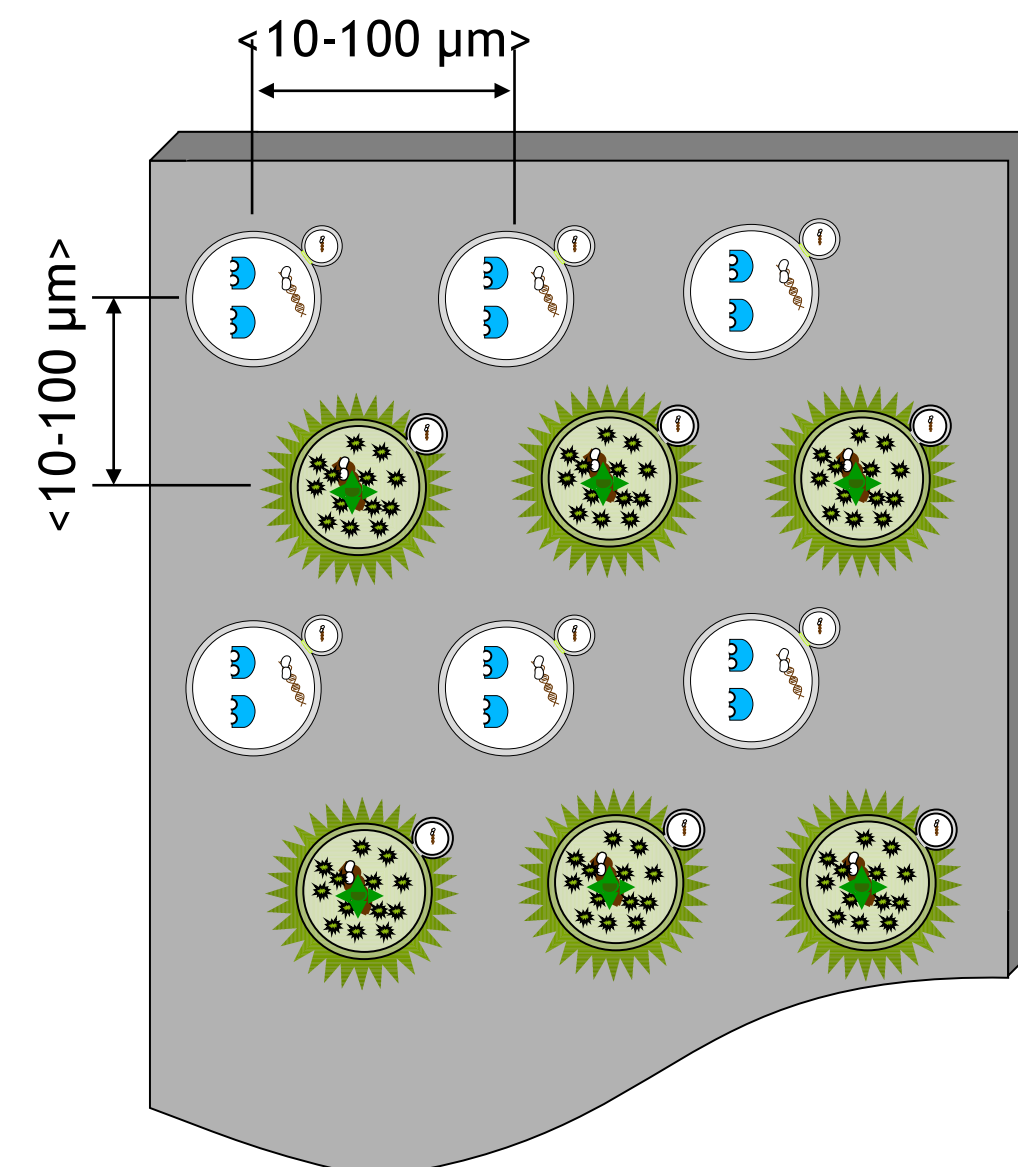


Analyst/ Stimulant Sensor cell α -factor Actor cells

Schematic illustration of yeast communication, where first cell senses an analyst and releases alpha factors to actor cells which in this case respond by fluorescence (example of yeast modified to express eGFP)

Concept

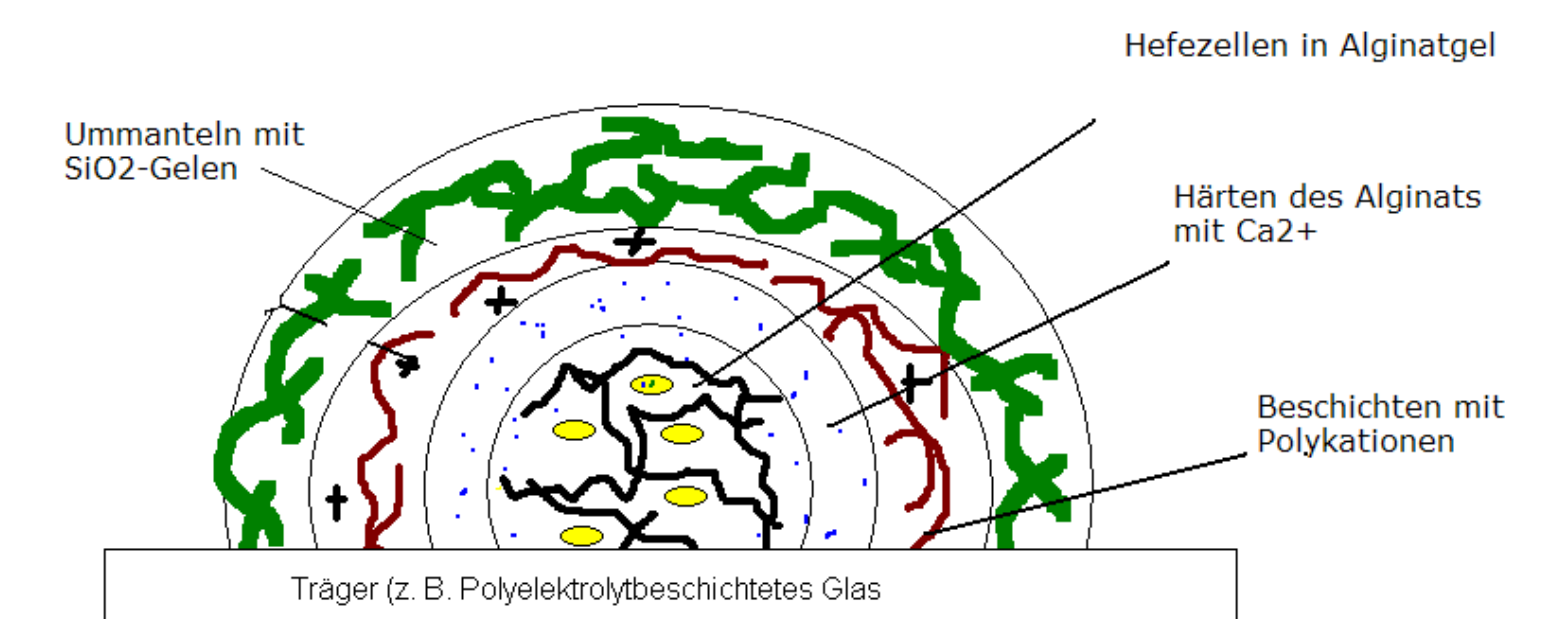
The volume of a single active cell unit has to be about 1 nL. The placement of the cells on the matrix is very important to avoid cross-talks as well as to allow an optimized design for coupling the biological components with a physical transducer.



For the intended sensor-actor system the patterned deposition of cells with defined distance in micrometer ranges is required. The patterning can be done by nanoplotting nanoliter volumes of cells immobilized in calcium alginate beads on a functionalized glass substrate with high hydrophobicity (contact angle about 140°).

Composition of sensor beads

- Yeast cells (different strains, e.g. BY 4741 with the gene for eGFP continuously expressed) were dissolved in 1.5 % sodiumalginate (medium viscosity). The solution was made 10 % with dextrane.
- The solution was deposited via the nanoplotting procedure on pretreated glass-slides (e.g. polycationic pretreatment).



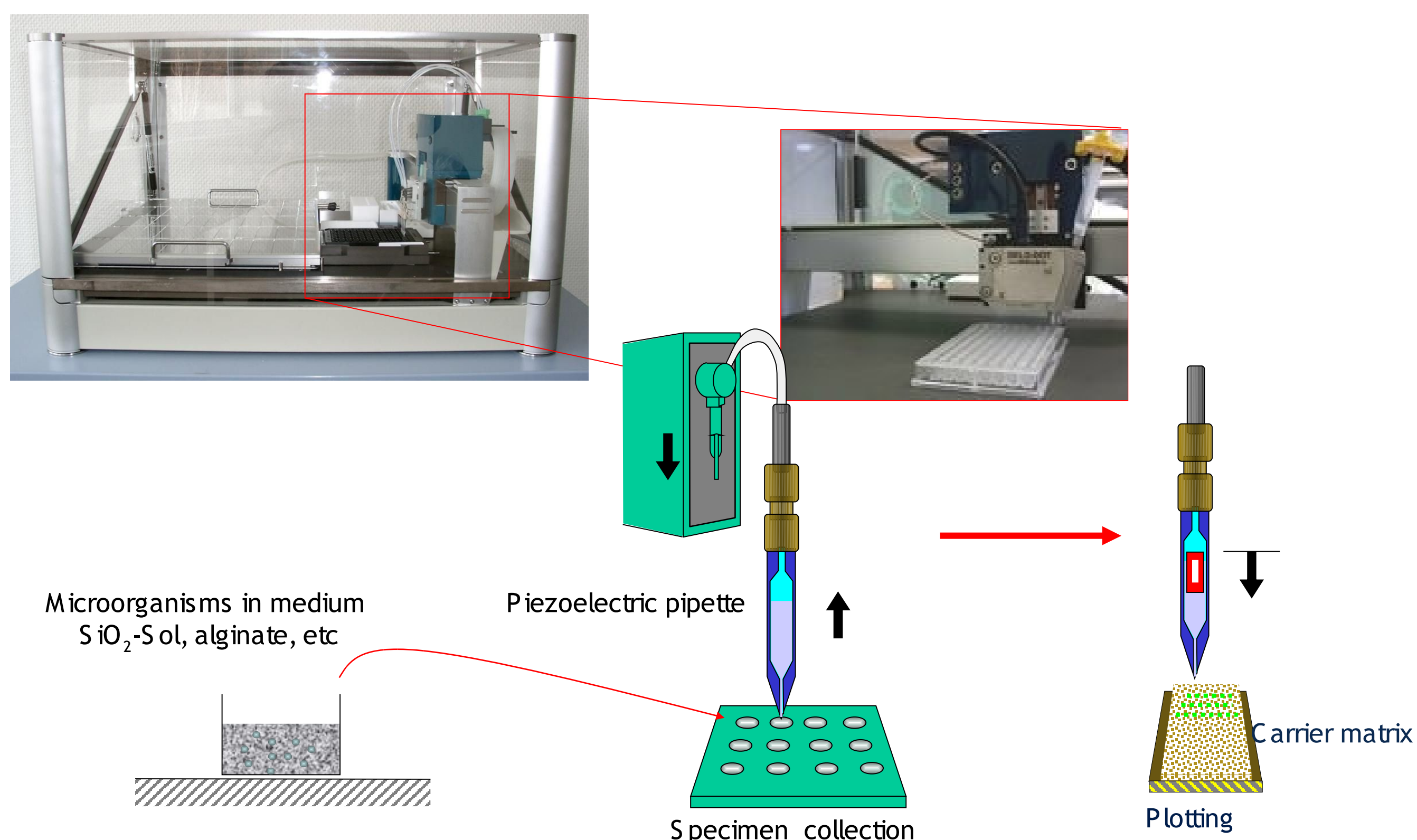
- After deposition the alginate is hardened with 0.1 M CaCl_2 for 20 min. A polycation is added to the hardening solution to enhance the strength of the coating layer. Finally, the beads were covered with SiO_2 sol/gel via dip-coating.
- After drying the objects are stored in 0.9 % sodium chloride.

Approach

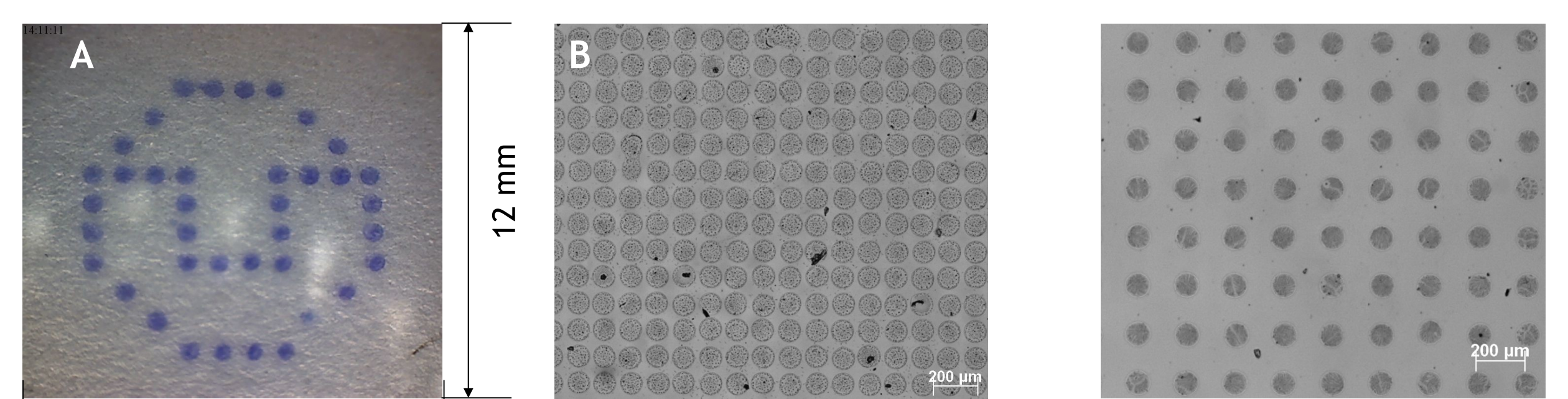
Nanoplotting procedure

Nanoplotter developed by GeS im mbH

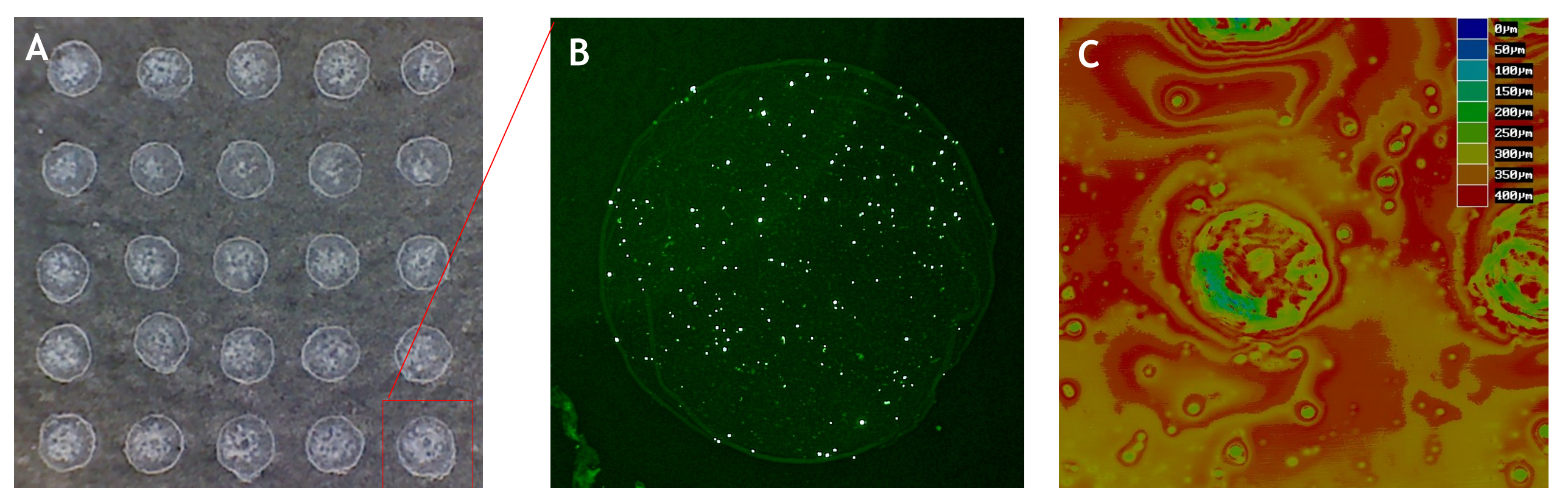
- a modular and flexible automatic pipetting system
- for sub-microliter dispensing and arraying applications.



Plotting results

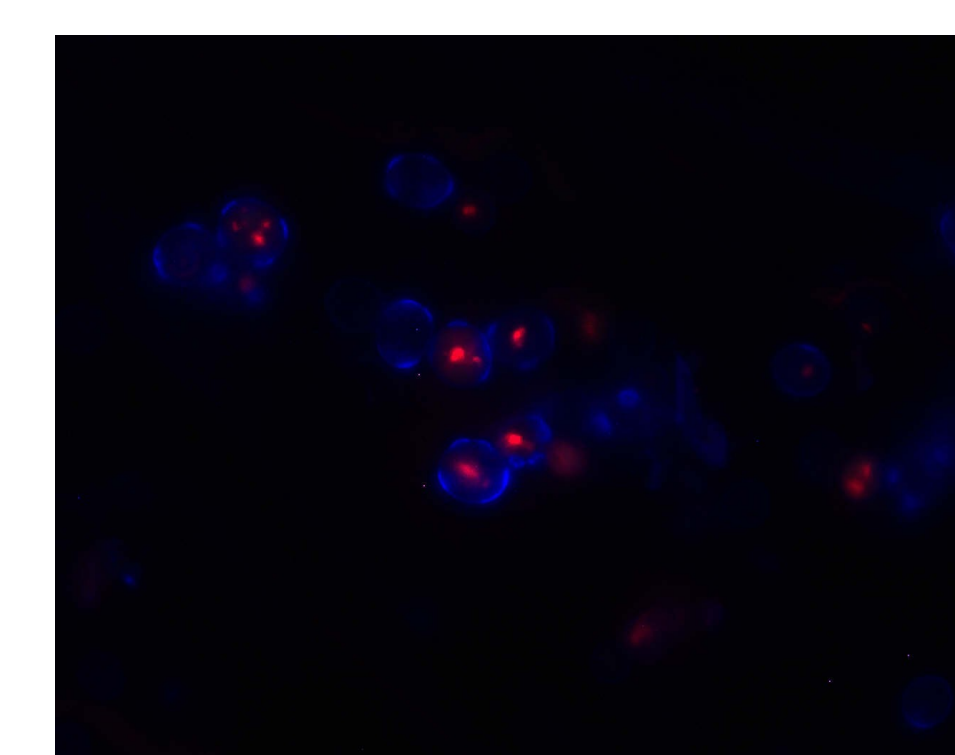
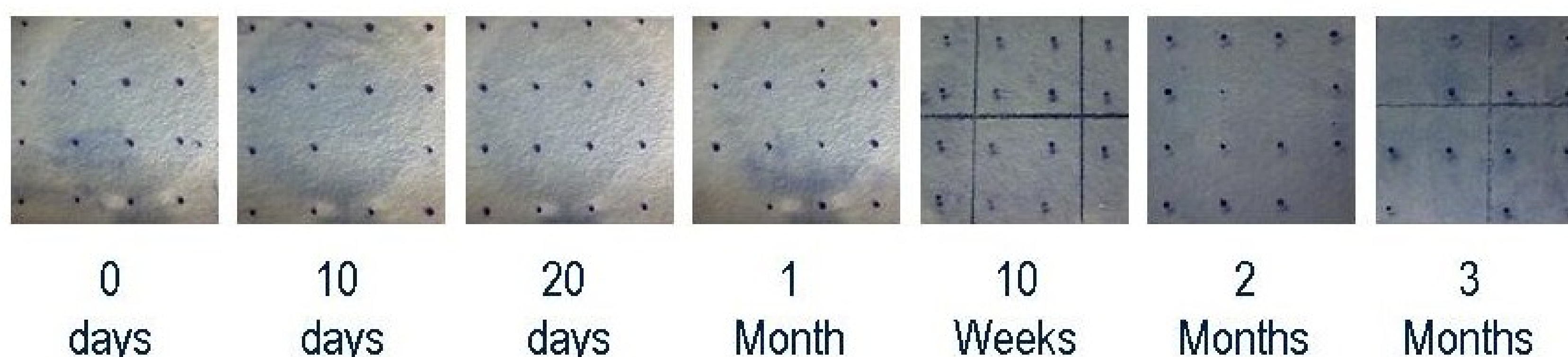


Nanoplotted structures: (A) The nanoplotter can be programmed to plot defined patterns; (B) A eGFP solution (80 μl) is deposited on a hydrophobic substrate (distance of the beads is about 20 μm), and (C) A eGFP solution (80 μl) is deposited on a glass slide



Nanoplotted recombinant yeast cells on silica-based matrices: (A) Microscopic view of nanoplotted alginate beads encapsulating recombinant yeast cells; (B) Fluorescence microscopy of a single alginate bead with yeast cells; (C) Height profile of the alginate beads.

Mechanical and biological stability



Nanoplotted yeast cells in alginate on silica-based matrices: After 2 weeks of storage in 0.9 % NaCl the yeast was inspected by life/dead probes (Calcofluor (blue) stains every cell, FUN 1 (red) shows the metabolic activity). Counting yields a living fraction of 50 %.

Nanoplotted recombinant yeast cells in alginate on silica-based matrices: slides were stored under current stirring (50 rpm) in 0.9 % NaCl. Probes were drawn after the given times, and the spots were stained with methylene blue for better visibility

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Conclusions and Acknowledgements

The nanoplotter technology allows placing beads from alginate 0.1 nL to as large as 100 μL . Therefore, these preliminary results entail that nanoplotting technology can be applied on specially functionalized surfaces and facilitates to plot precise distances as well as low volumes.

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