csem centre suisse d'électronique et de microtechnique

SIXTH FRAMEWORK PROGRAMME

hydromel Microrobotics & Self Assembly

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Fully Automated Approach for a High Throughput Microinjection System

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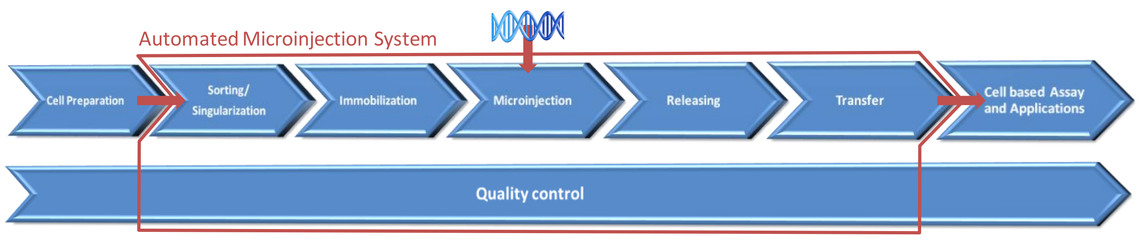
An innovative system to meet the need of drug researchers is developed in this project. The novel approach integrates concepts from microrobotics and microfluidics to achieve a high throughput (HT) device which will replace time consuming and costly manual operations used today. The complete cycle of manipulation for individual cells in suspension from sorting/singularization over immobilization, microinjection and releasing up to quality control will be regarded. Therefore in an intermediate stage a multipurpose robotic system called 'CellBot' was developed. In conjunction with a HT microinjection system the CellBot delivers single viable cells out of a suspension on demand to the microinjection system by applying a vision algorithm for the quality control of size and shape. The current microinjection system is designed for Xenopus oocytes but should also be suited for other large cells. The automated microinjection cycle for one large cell out of a suspension can thus be reduced from 300 seconds (manual) to 6 seconds (automated).

Introduction

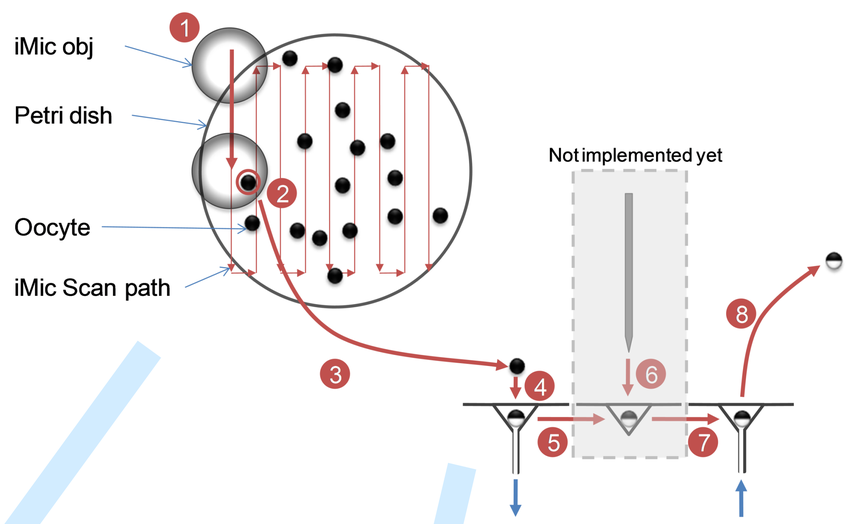
Microinjection into large cells like *Xenopus laevis* oocyte is a common transfection method in the field of drug discovery. However the drawback of this transfection method is its low throughput, costly manual operation and operator dependent success rate.

Therefore a fully automated microinjection system is developed within the EU-project HYDROMEL. The complete cycle from sorting/singularization, immobilization, injection, release to transfer will be integrated.



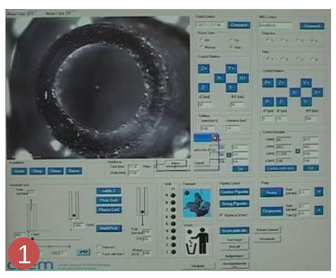
Challenges for the automation are:
- Sorting and singularization of cells out of a suspension
- Correct orientation of the oocytes (for later injection into the nucleus)
- Reliable immobilisation
- Cell adhesion
- Quality control

The intermediate concept



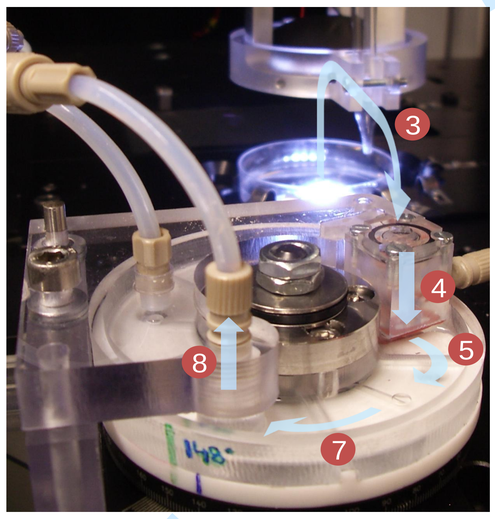
In an **intermediate solution** a robotic setup called 'CellBot' is used for sorting by a vision algorithm and singularization by vision feedback ②. The single cell is then transferred into the injection system ③, where the cell orients itself ④. The cell is then immobilised into a cone by gravitation and negative pressure and moved to the injection site ⑤ and later moved to the outlet ⑦ where the cell is ejected for the transfer into the storage vessel ⑧.

Results & Discussion



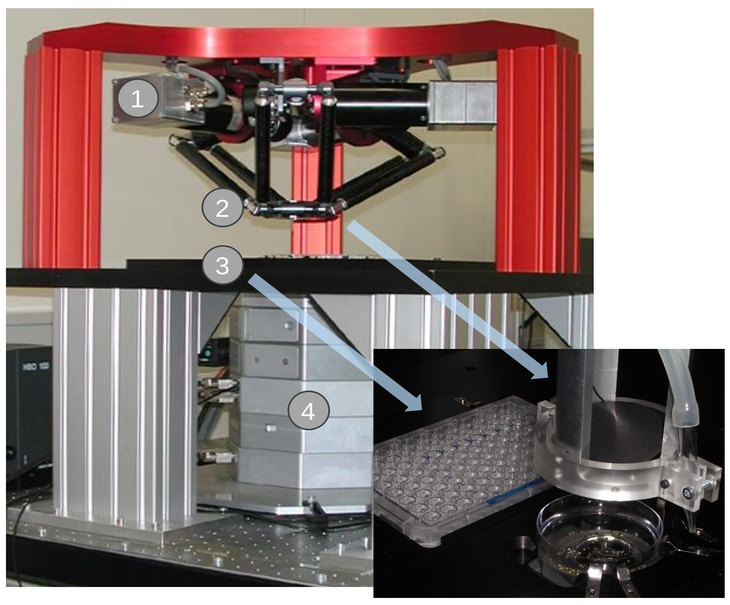
Autofocus onto glass capillary for later vision feedback

Scanning of the Petri dish until cells with correct shape and size are found. Then the glass capillary approaches the cell guided by vision feedback, the cell is removed and delivered into the immobilization system.



The integrated system allows sorting of single cells out of a suspension according their size and shape. By having a free fall length of 20mm the oocyte orients itself due to the density difference of its two poles. The carousel principle allows the three actions simultaneously: immobilisation in one site, injection in another site and releasing in again another site. The presented system reduces the 300s per oocyte manual injection time down to less than 6s per oocyte.

Sorting/Singularization System 'CellBot'



① µDelta (CSEM SA)
3 cycle/sec
<5 µm precision

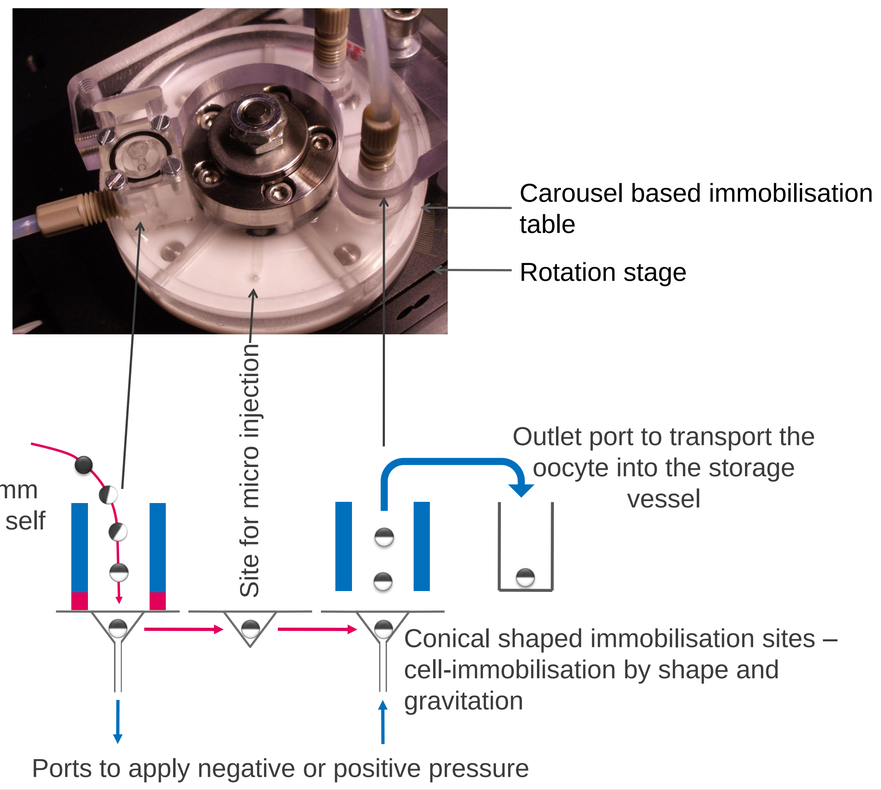
② Tool stage (CSEM SA)
for a 1.5mm glass pipette and a Peristaltic pump actuator

③ Working area (CSEM SA)
for a Petri dish, a multi titer plate (MTP) and the microinjection system

④ iMic (TillPhotonics GmbH)
fully automated inverse light microscope
BF, FL
Obj. 2x, 10x, 20x, 40x
X-Y-stage, Z-focus

CellBot system with close-ups of a glass pipette hold by the tool stage and the working area assembled with a Petri dish (filled with oocytes) and a multi titer plate – the injection/immobilization system is not shown.

Immobilization System



Outlook

For the **final solution** a low cost flow cytometer for large opaque cells to replace the CellBot, a force feedback for quality control of the microinjection step as well as a similar system for small adherent and suspended cells are in development. Finally the integration of all these techniques will lead to a complete low cost automated system for handling individual cells in a high throughput manner.

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