muTish—Towards a Cell Sorter for Medium Sized Biological Entities

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With the demand to replace, reduce and refine (3Rs) animal testing, higher quantities of 3D human tissue models will be used in future drug development and toxicity testing. These tissues are too large for conventional flow cytometers and therefore currently have to be manually manipulated. CSEM is thus developing an automated system, which characterizes and sorts individual spheroid tissue models in a size range of 200 to 500 µm. In addition a smart well plate is developed for long-term monitoring pH, lactate and glucose levels of such microtissues.

With this project CSEM's CellFactor technology was extended towards medium sized cells/cell clusters. In the past, this technology was designed for large objects in the size range of 500 to 2000 µm e.g. Zebrafish eggs or Xenopus oocytes and used viscous drag forces for transportation (patented). The extension towards 200 to 500 µm sized samples enables to handle biological entities such as microtissues or cell-spiked alginate beads. Conventional cell sorters do not cover these size ranges, because they are designed for sorting single cells, up to few tens of micrometers in size. Furthermore, they are built for very high throughput which is most often not a hard requirement for larger entities. Finally, conventional cell sorters can have low yields and typically require a huge number of cells to start with. In contrast, CSEM's CellFactor technology is designed to work with only a few up to several thousand entities and can sort them all reliably without loss. Real-time image based analysis and/or fluorescent detection can be used as a sorting criteria. To offer a robust fluidic setup, sensors for in-line process control are also developed. Additionally, to offer the complete cycle of analysis, a smart well plate is being developed which allows long-term monitoring of pH, lactate and glucose in each individual well.

The fluidic system (see Figure 1) is designed such that the samples are cycled between two sample containers. In-between the two containers, an image based inspection or fluorescent analysis of the samples takes place. If a sample fits the predefined sorting criteria, a small crossflow of about 20 µl ejects the sample into a well plate. The cycling offers a continuous analysis until all entities have been sorted.

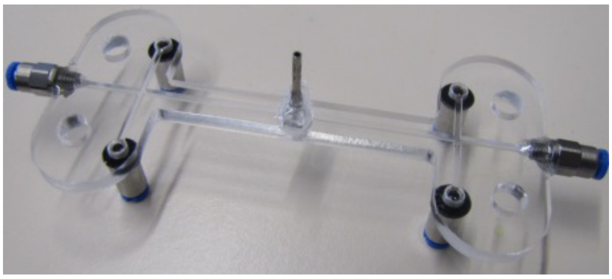

Figure 1: Symmetric flow cell for continuous measurements.

To keep the cost of the system low, the fluorescent module is designed to work with high power LEDs, a highly sensitive photo diode and dedicated optics to measure the very weak signals. Figure 2 shows the module currently optimized for green fluorescent signals (Fluorescein).

To further increase process stability of the fluidic setup, a sensor which measures the impedance of the passing entity is in development. In the lower MHz range, the differences between various cells and air bubbles are detectable. Figure 3 shows a first design of the inline detector.

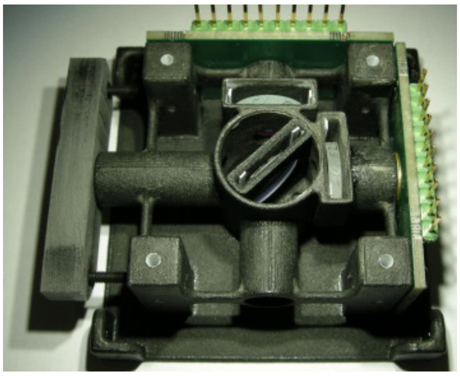

Figure 2: Cost-efficient fluorescence module.

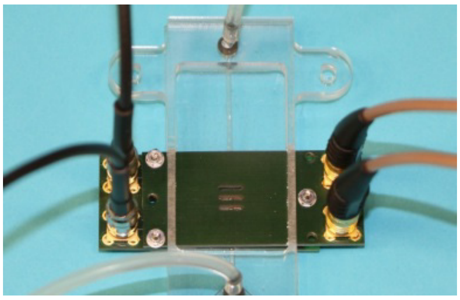

Figure 3: Inline sensor discriminating between air, bubbles and cells.

The smart well plate (see Figure 4) for subsequent long-term monitoring is thermoformed from PMMA substrates with pre-printed electrodes. The electrodes are bio-chemically modified with stable functional layers sensitive to pH, lactate or glucose, to provide long shelf-life and stable monitoring. In parallel to the development of the functional layers of the sensors, a new concept for a printed, solid state reference electrode is being developed to minimize the drifts.

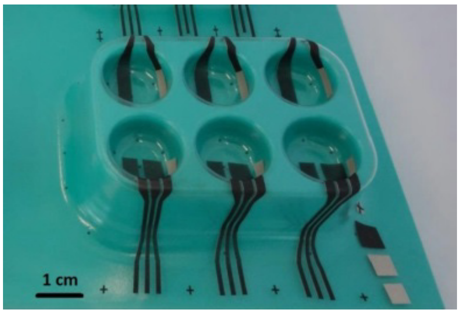

Figure 4: Thermoformed smart well plate with printed electrodes.

The technologies described were designed in close contact with our lead users hepia and fGen which work with neurospheres and alginate beads, respectively. In a next step, the novel cell sorter will be tested and evaluated with our lead users.

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