Microchip-based sorting of high-risk material such as bacteria, yeast, and parasites in a disposable and fully closed cartridge system using the MACSQuant® Tyto® Sorter

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Introduction

Microorganisms, particularly bacteria and yeast, are used by many scientists as a tool for molecular biology research. In contrast to mammalian cell systems, the speed of bacterial growth allows isolation of DNA and vast amounts of proteins within hours. Yeast cells are often used to study the localization and function of proteins using fluorescent gene reporter constructs.

Another group of organisms labeled under high-risk material are parasites. A commonly known example of parasites is *Plasmodium,* which causes malaria. The disease is initiated by the bite of a *Plasmodium*-infected mosquito, delivering sporozoites into the human host. Sporozoites are the "free" stage of the *Plasmodium* parasite. They infect liver cells, where they mature and multiply. Liver cells rupture and release the so-called merozoites, which then infect red blood cells. In red blood cells more merozoites are formed, leading to a continuous release of the parasites, which ultimately cause the malaria symptoms. Isolation of sporozoites has been proven problematic on conventional droplet sorters due to the harsh conditions such as high sorting pressure, the charge that is applied, and decompression.

Oftentimes, flow core facilities hesitate to conduct sorts of high-risk material on their conventional droplet-based systems due to the risk of sample-to-sample cross-contamination. It is difficult and time consuming to completely sterilize a conventional droplet sorter and the surrounding environment after use.

The MACSQuant Tyto (fig. 1) is a benchtop microfluidic sorter equipped with 3 lasers allowing for 10-parameter cell sorting. A unique feature of the instrument is the fact that the actual sorting process takes place exclusively within the MACSQuant Tyto Cartridge. This cartridge provides a fully closed, single-use system, eliminating the risk of sample contamination, carryover, and the generation of biohazardous aerosols.

Here we demonstrate the capacity of the MACSQuant Tyto to sort bacteria and yeast to high purities without the risk of sample-to-sample cross-contamination and extensive cleaning procedures in between.

In contrast to droplet sorters, sorting on the MACSQuant Tyto happens with a low air pressure of <3 psi applied to the cells. Moreover, sorting occurs without decompression, and no charge is applied. Therefore, the MACSQuant Tyto enables sorting of delicate stages of parasites, such as the sporozoites, without affecting viability and motility of the cells. As a proof-of-principle, sporozoites of the harmless *Plasmodium berghei* species were sorted to high purity.

Figure 1

Methods

1 The MACSQuant Tyto Cartridge

Figure 2A and B show the side view and bottom view of the cartridge, respectively.

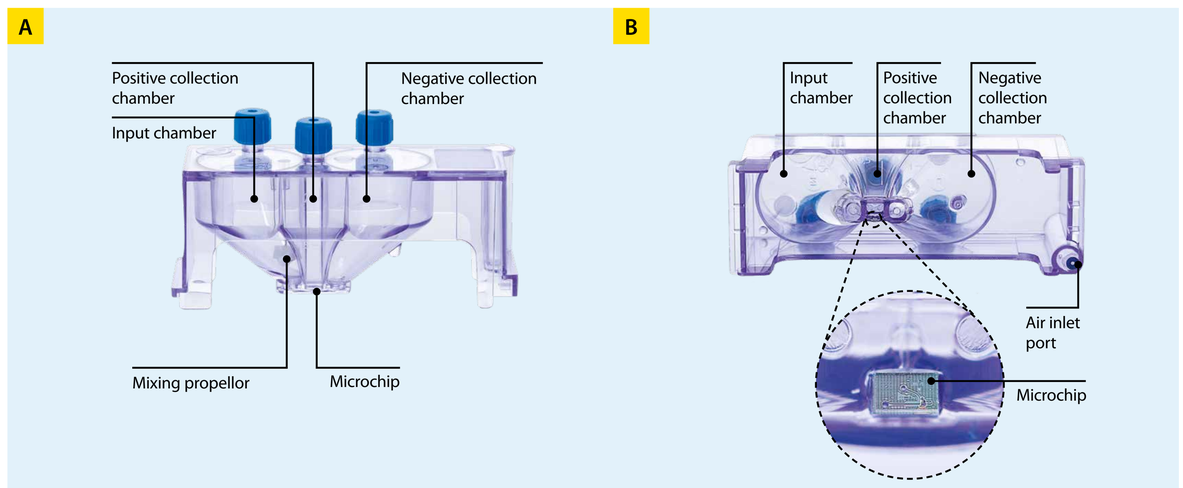


Figure 2

2 The sorting principle of the MACSQuant Tyto Cartridge

I. A single-cell suspension is loaded into the input chamber of the cartridge (fig. 3). The cartridge is placed into the MACSQuant Tyto Instrument. Filtered air coming from the instrument enters through a 0.1 µm filter within the air inlet port at the bottom of the cartridge and flows through the air pressure line towards the input chamber. The air enters the input chamber through another 0.1 µm filter, driving the cells through a microchannel into the microchip at very low pressure (<3 psi). Before entering the microchannel, potential cell aggregates are held back by a filter system guaranteeing a smooth sorting process.

II. Within the microchip, cells are interrogated by three lasers. Based on their fluorescent and scatter light signatures, target cells are redirected by a sort valve located within the microchannel (see magnification in fig. 1B). The default destination of cells is the negative collection chamber.

III. Once a target cell is identified, a magnetic field is applied to the microchip. This triggers the sorting valve to open and therefore redirects the target cell into the positive collection chamber. After a positive cell has passed through, a silicon spring returns the valve into its original position.

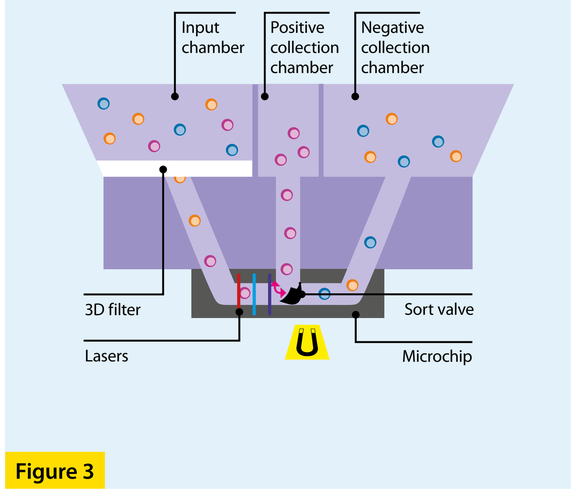


Figure 3

Results

1 Sorting of bacteria without cross-contamination, no need to completely sterilize the instrument

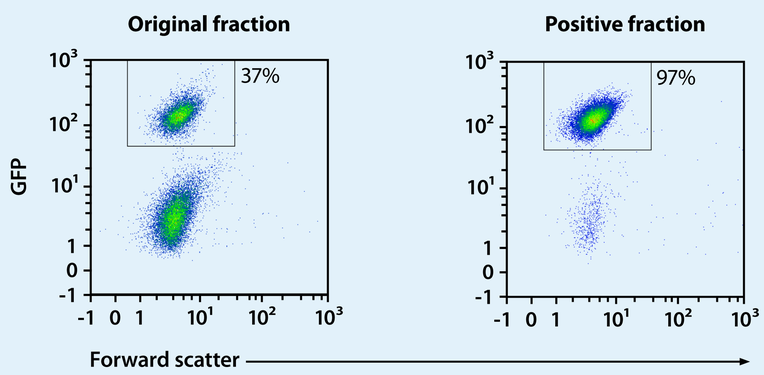


Figure 4

Using the MACSQuant Tyto, GFP-expressing *E. coli* were sorted to 97% purity from a mixture with wild type *E. coli*. The different fractions were analyzed by flow cytometry (fig. 4) on the MACSQuant Analyzer 10 prior to (original fraction) or after sorting (positive fraction). Directly after sorting the GFP⁺ bacteria, a new media-containing MACSQuant Tyto Cartridge was inserted into the instrument and processed for 2 hours. A bioburden test afterwards showed no cross-contamination of bacteria to the second cartridge (<1 CFU/mL), even after 14 days of culture.

2 Tight cartridge allows complete containment of yeast cells

Saccharomyces cerevisiae cells (2×10⁶ cells/mL) were transfected to induce expression of c-Myc protein. From a target cell frequency of 35% in the original fraction, the c-Myc⁺ yeast cells were sorted to a purity of 95.3%. Figure 5 indicates the gating strategy and designated sort gate.

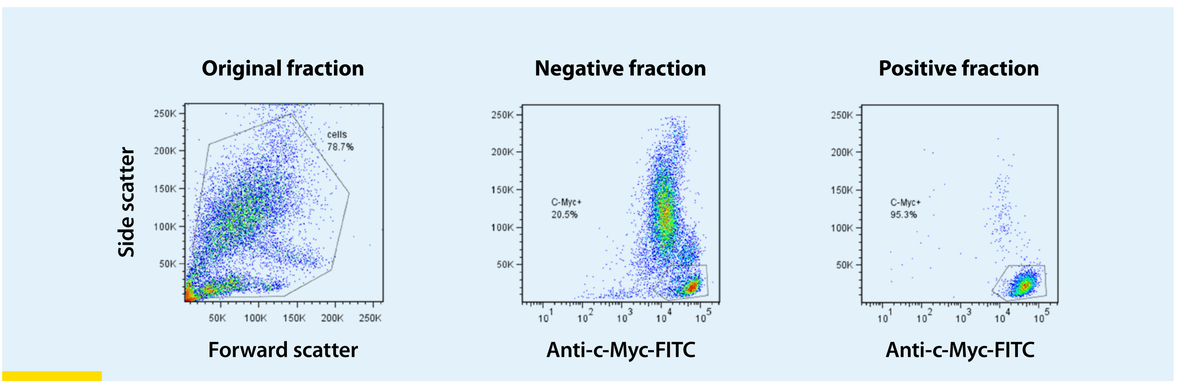
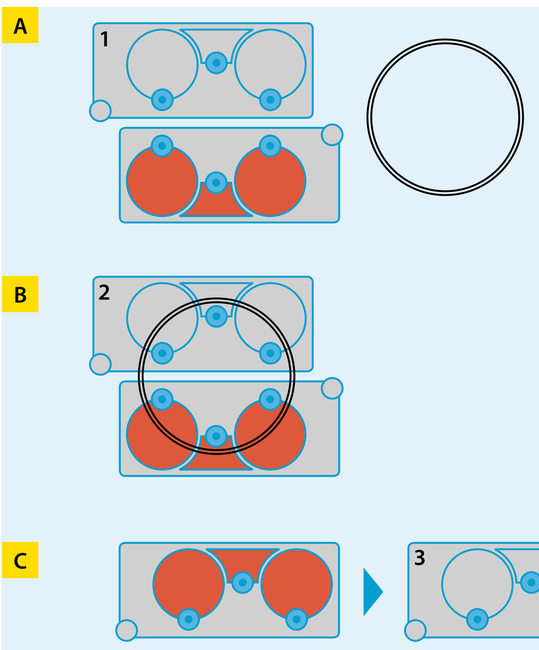


Figure 5

To investigate the tightness of the MACSQuant Tyto Cartridge, a mock system mimicking the sorting conditions within the MACSQuant Tyto Instrument was established, which allowed for pressurizing and stirring of the input chamber and for running fluid through the microchip. Two MACSQuant Tyto Cartridges were loaded with 7×10⁶ yeast cells/mL and a mock sort was executed. Adjacent to these cartridges, two cartridges containing only MACSQuant Tyto Running Buffer were positioned (fig. 6A and B, cartridges 1 and 2). To test for potential cross-contaminations, the running buffer of these cartridges was subjected to a bioburden test after the mock sort. No yeast cells could be detected after 9 days (<1 CFU) of cultivation of this medium. Additionally, agar plates were strategically positioned at the ventilation ports of the collection chambers and around the cartridges during the 90-minute sort to identify any yeast cells escaping the MACSQuant Tyto Cartridge. However, no yeast cells could be detected outside of the cartridge.

In an additional test, a MACSQuant Tyto Cartridge containing yeast cells was processed on the MACSQuant Tyto, followed by a cartridge containing running buffer (fig. 6C, cartridge 3). A bioburden test of the running buffer reconfirmed the safety and tightness of the MACSQuant Tyto Cartridge.



Agar plate	For 90 minutes located next to	Bioburden (after 9 days)
Plate 1	No cartridges (control)	<1 CFU
Plate 3	Yeast-containing cartridge while stirring without pressure	<1 CFU
Plate 5	Yeast-containing cartridge while sorting	<1 CFU

Agar plate	For 90 minutes located on top of	Bioburden (after 9 days)
Plate 2	Yeast-containing cartridge while stirring without pressure	<1 CFU
Plate 4	Yeast-containing cartridge while running	<1 CFU

Cartridge	Content	Bioburden (after 9 days)
1	Tyto Running Buffer	<1 CFU/6 mL
2	Tyto Running Buffer	<1 CFU/6 mL
3	Tyto Running Buffer	<1 CFU/6 mL

Figure 6

3 Gentle process of the MACSQuant Tyto enables sorting of delicate stages of *Plasmodium* parasites

Isolation of the sporozoite stage of the *Plasmodium* parasite is problematic on conventional droplet sorters due to the harsh sorting conditions. Using the gentle sorting conditions of the MACSQuant Tyto, 2.8×10⁵ GFP⁺ *Plasmodium berghei* cells/mL were sorted from a target cell frequency of 30% in the original fraction to 98% purity in the positive fraction (fig. 7).

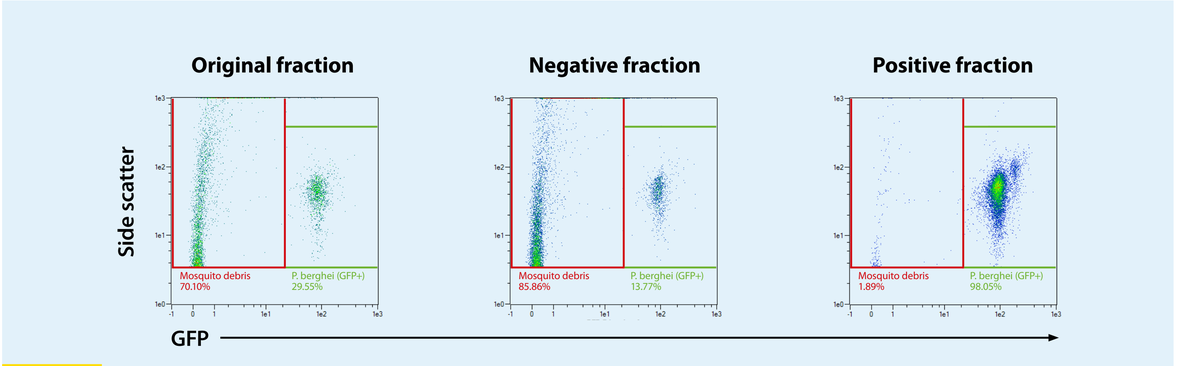


Figure 7

Microscopic images recorded with a time delay between fluorescence and bright-field image revealed movement of sporozoites directly after sorting on the MACSQuant Tyto, suggesting maintained viability of the sporozoites (fig. 8).

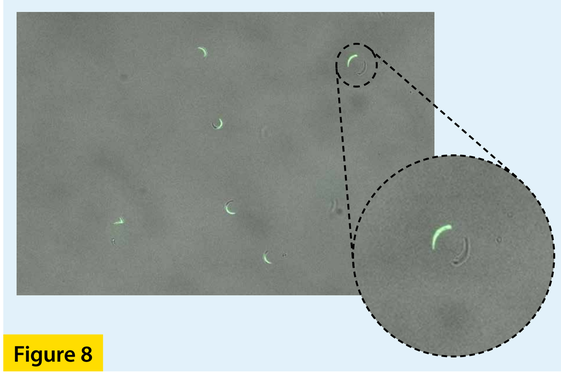


Figure 8

Conclusion

- We present a novel microchip-based sorting technology that allows for isolation of high-risk organisms such as bacteria, yeast, and parasites in a fully closed system.
- Cross-contamination and tightness tests performed using yeast, showed that all cells were retained in the closed MACSQuant Tyto Cartridge. No sample-to-sample carry over occurred.
- The gentle sorting conditions of the MACSQuant Tyto allow for sorting of delicate cell types such as the sporozoite stage of *Plasmodium* parasites. Sorted parasites were motile, which indicates that viability was maintained.