Real-Time Bacterial Capture and Sorting Using Dielectrophoresis

S. Simon[*], H. Markewich[**], E. Mallick[***], and M. Weber[****]

[*]Fluid-Screen, Inc.
700 Main St at Lab Central, Cambridge MA 02139 USA, xu.simon@fluid-screen.com
[**]Fluid-Screen, Inc. helen.markewich@fluid-screen.com
[***]mallick.emily@gmail.com
[****]Fluid-Screen, Inc., monika.weber@fluid-screen.com

ABSTRACT

Fluid-Screen, Inc. presents a bacterial capture and sorting method that bypasses the need for time-consuming culture-based methods of bacterial sample preparation. When combined with current rapid bacterial identification methods, the Fluid-Screen sample sorter has potential to reduce the time it takes to detect bacterial contamination from days to minutes. The technology has applications in biodefense; the municipal, recreational, and environmental water industries; clinical diagnostics; and sterile manufacturing quality control among other industries.

Fluid-Screen technology is based on dielectrophoresis (DEP) and is independent of bacterial growth. DEP has been known to influence particle motion and capture since the 1960's[1], however, technology based on DEP has historically had low efficiency, limiting its use for practical applications[2,3].

Fluid-Screen has developed a novel electrode that induces high electric field gradients to make use of DEP for bacterial capture[4]. The design of this sample-sorting electrode allows for rapid and efficient isolation of diverse bacteria from a variety of aqueous solutions[5]. Furthermore, by precisely controlling electronic conditions, the Fluid-Screen device can differentially process distinct types of bacteria for selective manipulation.

The Fluid-Screen sample sorter captures both Gram-positive and Gram-negative bacteria, along with those lacking a cell wall. Notably, it works with bacteria of a range of sizes[6]. Not only fluorescent laboratory-defined strains of bacteria, but also environmental bacteria, and bacteria endogenous to a sample respond to the electric field. Furthermore, bacteria behave differently from larger particles such as mammalian cells, opening the possibility of using the technology for sterilization or filtration applications to selectively remove bacteria from a contaminated source.

Bacteria are either attracted to or repelled from the Fluid-Screen sample sorter based on the applied frequency and voltage of the electric field, allowing for controlled capture and release. By selectively isolating and immobilizing bacteria from fluid, the sample sorter concentrates bacteria from a low-titer sample for low-volume release and transport into any number of bacterial detection or identification systems. This allows for real-time detection of bacterial contamination without time-consuming amplification methods. Although current Fluid-Screen designs are optimized to capture bacteria, the electrode feature design may be customized to capture and manipulate other particles such as viruses, proteins, and chemicals for efficient sample preparation prior to detection.

Keywords: dielectrophoresis, electroosmosis, microelectronics, microfluidics, microbiology

1 UNIVERSAL CAPTURE OF BACTERIA

We have previously shown simultaneous capture of Gram-positive and gram-negative bacteria[5], along with bacteria of small size or rod shape[6]. Here we show simultaneous capture of three organisms at once. The organisms are either Gram-negative (*E. coli*) or Gram-positive (*Enterococcus faecalis* and *Propionibacterium acnes*) and grow either aerobically (*E. coli* and *E. faecalis*) or anaerobically (*P. acnes*). Despite these differences, all bacteria are captured and aligned on the Fluid-Screen sample sorter as shown in Figure 1.

2 INDUSTRIAL APPLICATIONS OF FLUID-SCREEN TECHNOLOGY

Bacterial contamination poses risks in many industries, including both those such as pharmaceutical manufacturing that depend on complete sterility and those such as muncipal water processing for which only a certain type or level poses a threat. Fluid-Screen technology promises to inrease the speed with which contamination is detected, thereby saving time, money, and lives. Furthermore, the technology is compatible with passive in-line processes for real-time detection of contamination, or for use as a water filtration or purification method to ensure sterility.

2.1 Fluid-Screen in sterile applications

For sterile applications such as pharmaceutical manufacturing and food production, delays in detecting and identifying bacteria run the risk of spreading contamination to downstream processes or to end users. A simple yes-or-no answer to the presence of bacteria within minutes of

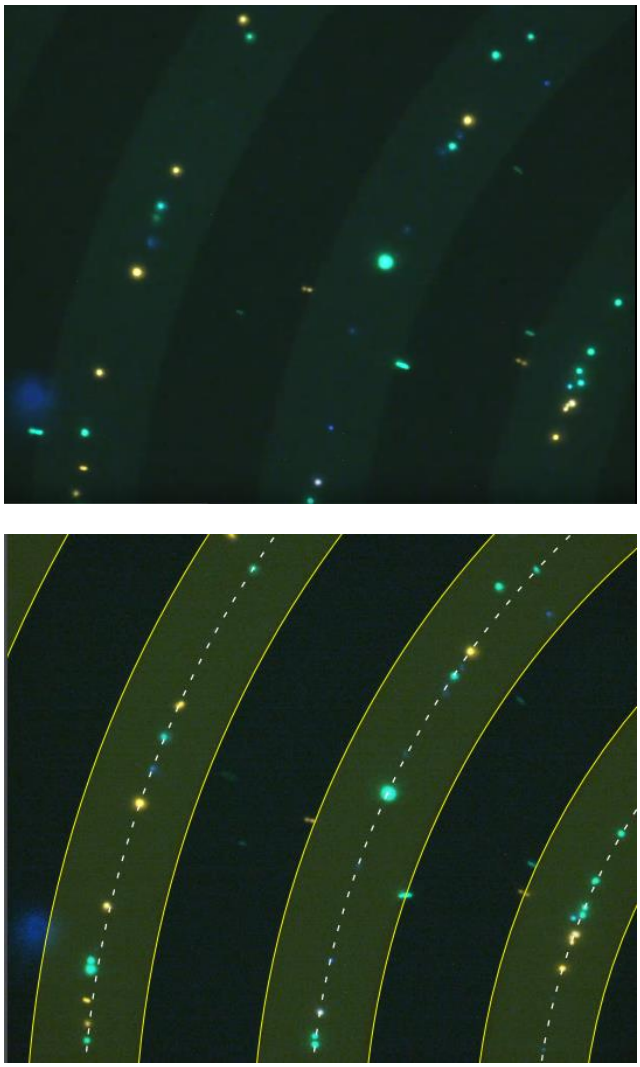

Figure 1: GFP-expressing *E. coli* (green), mCherry-expressing *E. faecalis* (yellow), and DAPI-stained *P. acnes* (blue) simultaneously align on the Fluid-Screen sample sorter for universal bacterial capture. Magnification, 60x. Top frame: direct visualization with fluorescent microscopy. Bottom frame: electrodes are outlined for visual clarity.

testing can alert users to the need for containment if a sterile environment becomes contaminated. This enables fast and efficient clean-up to prevent downstream contamination so that processes can get back on line quickly. Universal capture by the Fluid-Screen sample sorter ensures that any problem-causing bacteria can be captured and detected in time to avoid widespread contamination or contagion.

2.2 Specificity, selectivity, and quantification

For other applications, specific bacteria or bacterial loads may represent the biggest issue. For example, the Fluid-Screen sample sorter can be used to capture and concentrate specific microorganisms for testing and defense against bioterrorism agents. In non-sterile applications such as municipal and recreational water testing where the bacterial load is important, the Fluid-Screen sample sorter can use precise volumes to enable accurate bacterial counts in a batch system.

Fluid-Screen also shows promise for rapid medical diagnostics. In this use case, determining the precise type of bacterium or its antibiotic reistance markers is important for prescribing effective treatments. Current culture-based methods can take several days, imposing a monetary burden on both individuals and society while needlessly prolonging suffering for a patient awaiting diagnosis. Fluid-Screen rapidly prepares bacteria by concentrating them directly from the sample for processing and identification.

2.3 Accurate results for nonculturable bacteria

Among Fluid-Screen's great potential benefits is with slow-growing or unculturable bacteria. *Mycoplasma* contamination in manufacturing lines takes four weeks to detect due to the slow and sensitive nature of the *Mycoplasma* culturing process. By first concentrating bacteria into a small volume as a sample preparation method, the time consuming bacterial culture process is bypassed, and rapid methods of bacterial identification will alert the user to the presence or absence of the bacteria of interest. In this way, Fluid-Screen represents a rapid sample preparation method for existing rapid bacterial identifiction methods.

In emerging applications such as microbiome research and diagnostics, Fluid-Screen can specifically capture all bacteria while retaining their relative abundance. Culture-based methods bias analysis towards faster-growing bacteria, which may obscure the effects of important but slow-growing bacteria in microbiome samples. Fluid-Screen removes this bias by directly capturing bacteria rather than by relying on growth.

2.4 Custom sensing and filtration applications

Because the Fluid-Screen sample sorter can function in unversal bacterial capture or may be fine-tuned to slectively capture specific types of bacteria, the choice of modular detection unit becomes important in some applications. Fluid-Screen is compatible with any number of downstream detction methods.

With its use in universal bacterial capture, the Fluid-Screen sample sorter can either be coupled with a wide variety of rapid bacterial detection and identification units for array analysis, or it can function entirely on its own to remove bacteria from a sample and ensure sterility. This

latter application is akin to a sterile filtration unit with the advantages of flow at consistent pressure while efficiently capturing even very small bacteria.

When the electic field is fine-tuned to select only specific bacteria, the Fluid-Screen sample sorter reduces the potential noise inherent in very sensitive bacterial identification methods. Furthermore, by selectively concentrating only bacteria of interest, the Fluid-Screen sample sorter amplifies the signal of very selective identification methods.

2.5 Additional applications for Fluid-Screen technology

Because sample transfer to the electrode works with microfluidic flow, the technology is compatible with in-line continuous flow monitoring. For use in any sensing application, in-line flow enables passive monitorng for either specific bacterial contamination or for bacterial loads that reach a certain threshold. The user can confidently continue operations until the threshold is reached and an alarm signals the need for action.

3 CONCLUSIONS

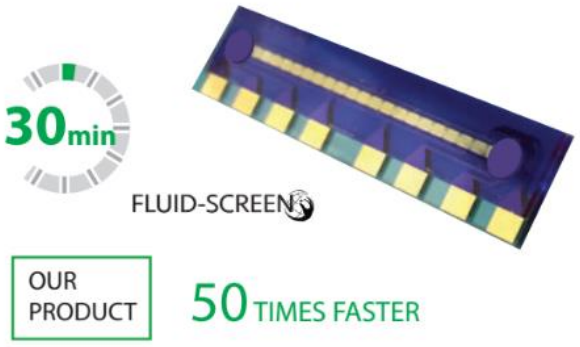

Figure 2: Manufactured prototype of Fluid-Screen sample sorter used for testing and filtration.

Fluid-Screen technology combines proven technology with innovative application to create a rapid sample preparation system for use with any bacterial detection or identification module. The system can be customized for universal bacterial capture or to provide a level of selectivity for capture of specific species. Coupling to a specific bacterial detector adds an additional layer of customizability; a universal Fluid-Screen electrode can be used with any number of specific detectors, whereas a more selective capture unit can reduce the number of false positives inherent in a sensitive bacterial detection system. In all applications, Fluid-Screen eliminates the need for time-consuming culture processes for sample preparation and aims to make 30 minutes the new standard in bacterial contamination detection.

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