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Broad-Range Bacterial Capture from Fluid-Samples: Implications for Amplification-Free Contamination Detection

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Abstract: Fluid-Screen, Inc. presents a bacterial concentration and filtration method based on dielectrophoresis and alternating current kinetics. Dielectrophoresis has been previously shown to induce particle motion; however, bacterial capture efficiency and reproducibility have consistently been low, reducing its potential for practical applications. In this study, we introduce a novel, patent-pending electrode system optimized to simultaneously capture a wide range of bacterial species from a variety of aqueous solutions. Specifically, we show that the method of dielectrophoresis used induces responses in both characteristic Gram-negative *Escherichia coli* and Gram-positive *Enterococcus faecalis* bacteria, as well as with *Bacillus subtilis* and *Aestuariimicrobium kwangyangense*. We have adapted the electrode design to create a bacterial sample preparatio unit, termed the sample sorter, that is able to capture multiple bacterial species and release them simultaneously for bacterial concentration and exchange from complex matrices to defined buffer media. This technology can be used on its own or in conjunction with standard bacterial detection methods such as mass spectroscopy. The Fluid-Screen product will dramatically improve testing and identification of bacterial contaminants in various industrial settings by eliminating the need for amplification of samples and by reducing the time to identification. *Copyright © 2016 IFSA Publishing, S. L.*

Keywords: dielectrophoresis, AC kinetics, microfluidics, bacteria, microelectronics, radio frequency, cell sorter, contamination.

1. Introduction

Most bacterial detection techniques currently in use are time consuming and require the organism to be cultured, isolated, and amplified. Moreover, these techniques pose the risk contamination, which lead to delays in the time to detection and bacterial identifcation. In environmental and municipal contexts, contaminants, especially potentially pathogenic bacteria, can pose a serious health threat to the public resulting in illnesses or even death [1]. In manufacturing contexts, undetected contamination travels to downstream processes, resulting in costly downtime and decontamination, as well as public health risks if the contamination event remains undetected before the product leaves the manufacturing facility. This can be an especially prevalent problem in the food and beverage industry as well. In medicine and diagnostics, current bacterial detection and identification techniques are far from

perfect, resulting in lost productivity and high costs of quality control processes.

Culturing infectious agents from human samples and obtaining a diagnosis takes time and many microbial pathogens are often missed in the process [2]. Furthermore, antibiotics are often perscribed prior to determining whether a patient has a bacterial infection, which contributes to the problem of antimicrobial resistance [3].

Bacterial detection often requires a culture-based method to enrich a small initial quantity of bacteria to a minimum yield for reliable analysis. This process requires at least 8 hours for the fastest-growing bacteria and up to several weeks for slow-growing contaminants such as *Mycoplasma sp*. Among the "rapid" methods of bacterial detection, Polymerase Chain Reaction (PCR)-based and sequencing-based techniques still require DNA amplification, often require culture-based sample preparation, and are expensive and technically involved [2]. Therefore, even the fastest microbial identification techniques require a minimum of hours to obtain results.

Here, we describe a novel, method of bacterial detection and identification using an automated, portable device for rapid pathogen sorting from fluids [4]. The device ("sample sorter") uses dielectrophoresis (DEP) to capture bacteria. Once concentrated from a fluid sample, bacteria can be detected using standard bacterial identification techniques, including the proprietary Fluid-Screen pathogen sensor designed to target either specific species of concern or a broad base of indicator bacteria. Additionally, overall bacterial loads can be determined using this technology.

DEP takes advtange of a particle's response to a non-uniform electric field [5]. DEP acts on particles, such as bacteria, by inducing a polarization gradient that generates controllable motion and attachment. Depending on the frequency of the applied field, the particles' Clausius-Mossotti (CM) factor, and the conductivity of the medium, DEP can attract particles to or repel them from a surface. Fluid-Screen has fine-tuned these parameters to control bacterial motion, as well as capture and release bacteria when appropriate in the assay.

Fluid-Screen has improved on previous electrode designs for DEP by creating a unique electrode that enables a steep electric field gradient [6, 7]. This design results in improved efficiency of bacterial capture, from about 70% in the previous state of the art [6, 7] to over 99%. This efficiency of capture makes the Fluid-Screen technology a valuable public health tool for water monitoring, waterborne disease prevention, sterility maintenance in manufacturing, food and beverage, and medical diagnostics.

2. Rapid Bacterial Capture

In the following examples, a bacterial suspension of 10^1 – 10^5 CFU/µL in defined Phosphate Buffered Saline (PBS) medium was applied directly to the sample sorter. These bacterial concentrations were chosen to show both a clear visualization under fluorescent microscopy conditions and to represent a wide variety of concentrations. Thus far, there has been no obvious effect of bacterial concentration on cell sorter performance. The electric field gradient was induced and bacterial motion and capture were visualized using epifluorescence microscopy recorded in real-time.

Repositioning of suspended bacteria was seen within milliseconds of applying an external electric field to the cell sorter. As shown in Fig. 1, captured bacteria collected onto the electrode and showed nearly complete capture within seconds of application of the electric field gradient.

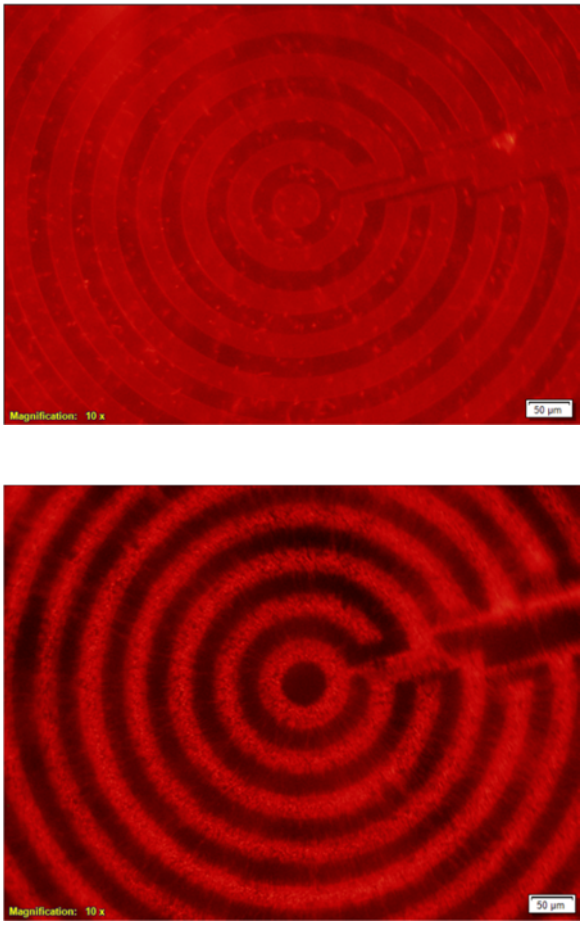

Fig. 1. *E. coli* expressing mCherry (red) and captured on the Fluid-Screen cell sorter at 10x magnification. Images were taken before (top) and 30 seconds after (bottom) application of the electric field. Note the uniform cell distribution before field application, and both the rearrangement and the increase in cell number 30 seconds after application.

3. Capture of Multiple Species

In addition to laboratory-standard *E. coli*, the Fluid-Screen cell sorter captures *E. faecalis* (Fig. 2), *B. subtilis* (Fig. 3), and *A. kwangyangense* (Fig. 4). These bacteria represent a range of sizes, with *A. kwangyangense* the smallest at 0.6 – 2.0 µm [8] and *B. subtilis* the largest at 4-10 µm [9].

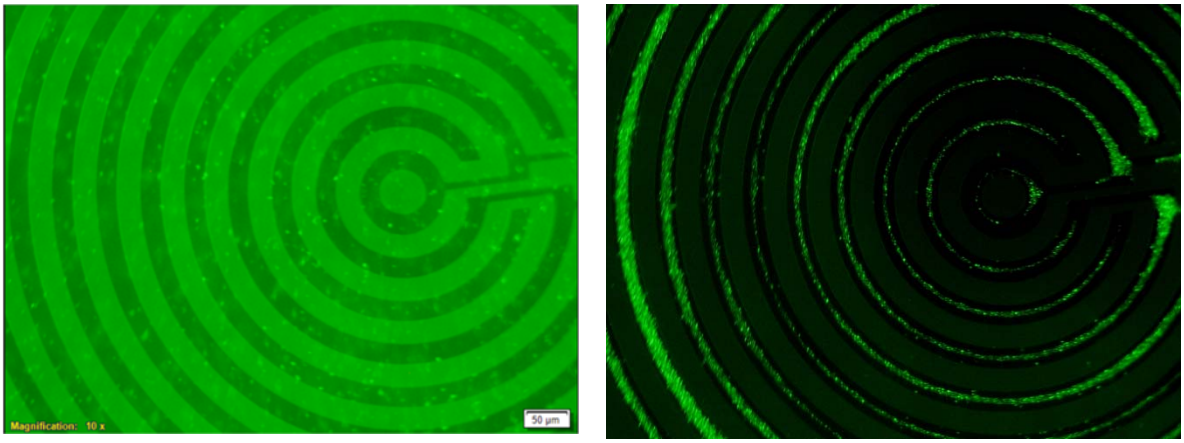

Fig. 2. *E. faecalis* expressing GFP (green) and captured on the Fluid-Screen cell sorter at 10x magnification. Images were taken before (top) and 30 seconds after (bottom) application of the electric field.

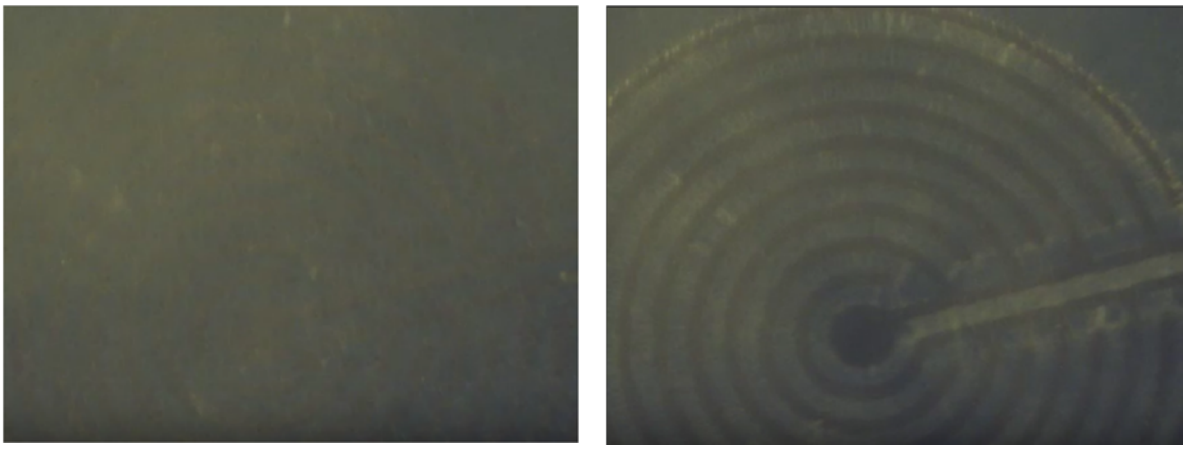

Fig. 3. *B. subtilis* expressing mCherry (red) and captured on the Fluid-Screen cell sorter at 10x magnification. Images were taken before (top) and 60 seconds after (bottom) application of the electric field. Notice both the collection of bacteria on the electrode and the structured alignment of the rod-shaped organisms after electric field application.

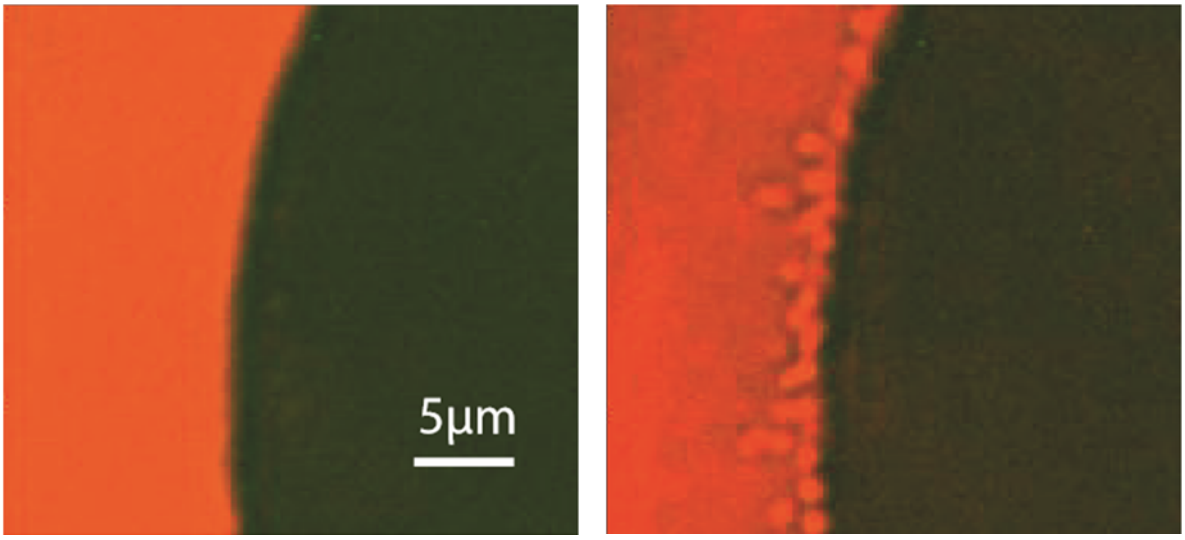

Fig. 4. NucRed Live 647-stained *A. kwangyangense* bacteria captured on the Fluid-Screen cell sorter. Photos show 60x magnification and were taken before (top) and 30 seconds after (bottom) application of the electric field.

4. Broad-range Bacterial Capture

The applied electric field on the cell sorter can be tuned for capture of more than one species at once. Fig. 5 shows simultaneous capture of *E. coli* (red) and *E. faecalis* (green). Broad-range capture of diverse bacteria allows for efficient detection of bacterial contamination in a variety of contexts, regardless of the identity of the contaminant.

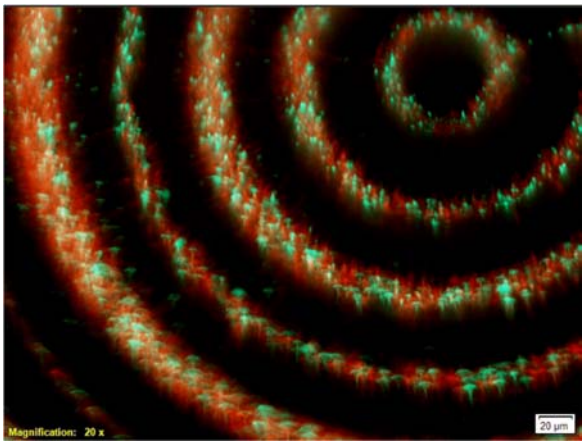

Fig. 5. *E. faecalis* expressing GFP (green) and *E. coli* expressing mCherry (red) captured simultaneously on the Fluid-Screen cell sorter at 30 seconds after application of the electric field. Magnification, 20x.

5. Bacterial Capture from Native Fluids

The Fluid-Screen sample sorter extracts bacteria from complex fluids in addition to defined laboratory-grade buffer sultions. Water gathered from the Charles River in Cambridge, MA was filtered to remove particulates, inoculated with exogenous fluorescent *E. coli* or *E. faecalis* bacteria, and applied to the Fluid-Screen sample sorter. Upon application of the electric field gradient, fluorescent bacteria migrated through the water to collect on the electrodes. Fig. 6 shows results of *E. coli* capture from riverwater, whereas Fig. 7 shows *E. faecalis* capture from the same matrix.

6. Potential Applications

Fluid-Screen technology presents a platform for efficient and broad-range bacterial capture and concentration with applications in diverse industries where bacterial contamination poses a threat. The Fluid-Screen sample sorter allows for simultaneous capture of Gram-positive and Gram-negative bacteria, and allows for their controlled release. By immobilizing essentially all bacteria in a sample onto the electrode and then releasing them simultaneously in a small volume of defined medium, the Fluid-Screen sample sorter can be used to rapidly collect bacterial contaminants for detection and identification using any current industrial standard technology, without the use of time-consuming amplification techniques to generate a readable signal.

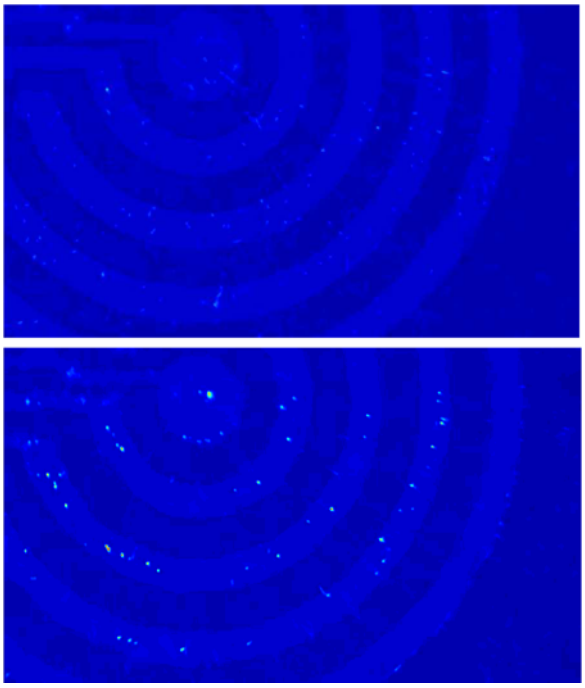

Fig. 6. *E. coli* expressing GFP (green) and resuspended in water from the Charles River in Cambridge, MA was captured on the Fluid-Screen cell sorter. Images were taken were taken before (top) and 30 seconds after (bottom) application of the electric field. Magnification, 10X.

The platform technologies has applications in industries including environmental, public water supply, sterile manufacturing such as pharmaceutical, water filtration and purification, point-of-care medical diganostics, agricultural, food and beverage. Fluid-Screen applies an improved dielectrophoretic filter design and bacterial identification modules in order to directly detect bacteria in fluid samples.

Future iterations on design and application will result in automated, integrated sample preparation and bacterial identification systems tailored to industry needs. The technology can be adapted to provide real-time, in-line continuous microbial monitoring along water lines and in fluid sources.

Finally, because DEP can be used to capture other particles in addition to bacteria, the electrode feature design and applied electric field can be optimized to manipulate other microscale contaminants.

Fig. 8 shows a schematic of the final, integrated Fluid-Screen microbial detector as envisioned for applications in sterility testing in the pharmaceutical industry. The sample enters the cell sorter presented here and a proprietary bacterial identification component. A sterile disposable chip inserts into a handheld Bluetooth-enabled portable reader. Microfluidic pumps process the fluid sample. After 30 minutes (assuming flow of 100 mL), results are transmitted to a smartphone or a central database.

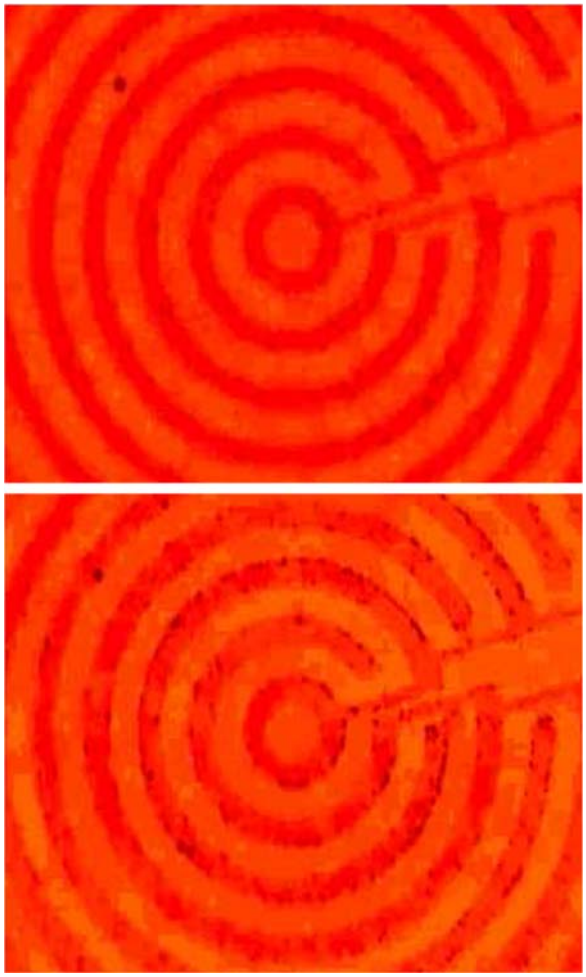

Fig. 7. *E. faecalis* expressing GFP resuspended in water from the Charles River in Cambridge, MA and captured on the Fluid-Screen sample sorter. Images were taken before (top) and 30 seconds after (bottom) application of the electric field. Magnification, 10X. Images were optically processed for visual clarity by attenuating the GB channels in the RGB image to emphasize red bacterial particles (dark spheres on the edges of the electrodes). Note the alignment of the bacteria on the electric field in the bottom picture, in spite of weaker fluorescence signal in river `water.

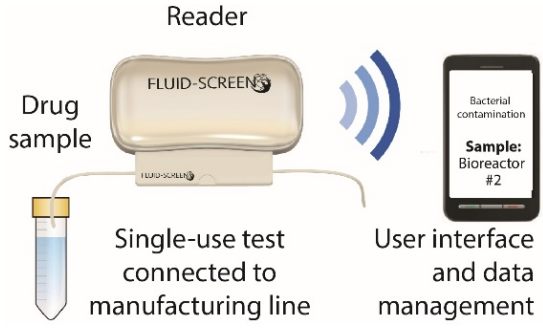

Fig. 8. Design of the optimized Fluid-Screen integrated bacterial detector unit, including the cell sorter presented here and a proprietary bacterial identification component. A sterile disposable chip inserts into a handheld Bluetooth-enabled portable reader. Microfluidic pumps process the fluid sample. After 30 minutes (assuming flow of 100 mL), results are transmitted to a smartphone or a central database.

7. Conclusions

Fluid-Screen, Inc. presents its patented technology for bacterial capture and concentration that enables rapid, portable, and accurate universal pathogen detection in a variety of fluids [10]. Pilot studies are underway for feasibility testing in multiple industries, and interested parties are encouraged to reach out regarding opportunities for creating customized bacterial detectors to fit their specific pathogen testing needs. The first commercially available manufactured prototype cell sorter devices will be available for purchase in early 2017.

Acknowledgements

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