The ToxiSense detection system: A novel centrifugal-based microfluidic (Lab-On-A-Disc) system for detecting cyanobacterial toxin microcystin-LR

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Abstract
With population growth, there is an ever-increasing requirement for fresh-water sources and safe, 'toxin-free' shellfish and fish stocks. To monitor these resources carefully, there is a need for the development of an *in-situ* algal-toxin monitoring solutions. Presented here is one of these solutions; *The ToxiSense Detection system* (Figure 1). This system has been designed to be as generically applicable to toxin detection as possible, with the cyanobacterial Microcystin-LR used for proof-of-concept.

KEYWORDS: Microcystin, Lab-on-a-disc, LOAD, Microfluidics, In-situ detection, Fluorescent detection.

Introduction
The most ubiquitous and dangerous cyanobacterial toxins are the microcystin family; a cluster of cyclic peptide toxins.(1) These algae have a tendency to bloom in either fresh or brackish water sources, with the notable example of Lake Erie, a fresh water source that is shared between Canada and the United States (2). Microcystin-LR is presently the most frequently occurring strain of the microcystin family. Microcystin-LR presents a major threat to marine resources and, thus, the EU have invested significant funding into investigating monitoring solutions for causative algal species. A target guideline value for Microcystin-LR in drinking water is 1 µg/L.(3) This sensitivity range makes microfluidics-based platforms ideal, and more importantly, when used with recombinant antibody technology, make this target achievable.

Hardware and Detection System
The complete detection system (Figure 1.a) consists of two main components; The ToxiSense system (Figure 1.b-c), and the ToxiSense disc (Figure 1.d). The 3d-printed ToxiSense system is designed to hold the ToxiSense disc, with a fluorescent detection apparatus incorporated to separately capture fluorescent values at both reservoir three and four on the ToxiSense disc, (Figure 1.d). The fluorescent label used is Alexa Fluor 647, manufactured by *Life Technologies*™, which has an excitation and emission of 650 nm and 665 nm respectively.

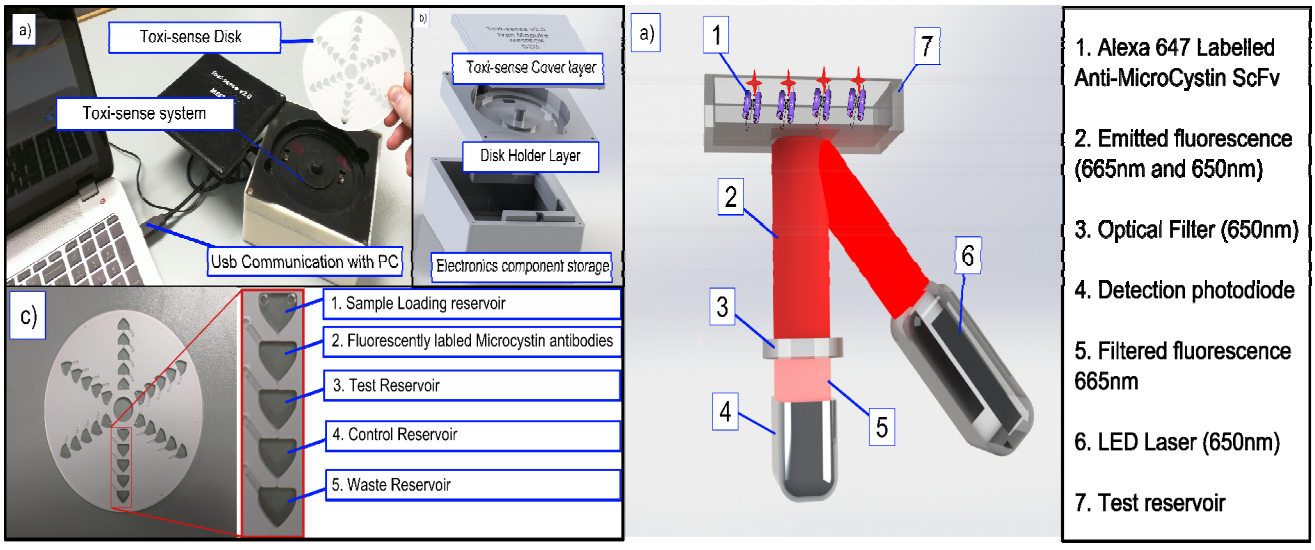

Figure 1: The ToxiSense system. Figures 1.a-b demonstrate the ToxiSense system. Figure 1.c illustrates the fluorescent detection set-up. Figure 1.d provides a reservoir breakdown of the ToxiSense disc.

The full detection model is illustrated in Figure 1c and 1d. The ToxiSense disc is manufactured from Poly(methyl methacrylate) (PMMA) sheets and Pressure sensitive adhesive (PSA) (*ARseal™90880*), sourced from *Radionics™* and *Adhesives Research™* respectively. The disc consists of five reservoirs, each with separate ventilation systems, in radial alignment connected by microchannels. This version of the disc is currently designed to be manually operated, with each ventilation hole initially capped. The sample will progress into the subsequent reservoir once the residual air can escape, thus only once the subsequent vent is opened. This is due to the sample effectively capping the air within the system by covering the microchannel. This ventilation system was required as the length of each step (5-10 minutes) was orders of magnitude greater than previous microfluidic valving solutions, such as the current dissolvable film valving solutions (<50 seconds).(4)

Assay integration and future work

Each reservoir on the disc represent a functional step in the assay, which is illustrated as an inverse assay using Microcystin-LR (Figure 2). Toxin detection takes place in reservoir three, where a low signal indicates a high concentration of Microcystin LR. A control was also added at reservoir four to confirm that fluorescently labelled antibody progression through the system was successful, with a waste reservoir added (reservoir five) for collection. This waste reservoir was incorporated to have a dry, non-submerged detection at reservoirs three and four. Figure 3 demonstrates the recorded fluorescent data of the ToxiSense system against varying Microcystin-LR concentration, with supporting fluorescent microscope images. The ToxiSense disc is currently being enhanced become a fully automated system (Figure 4), with a motor also being added to the ToxiSense disc holder for in-situ usage.

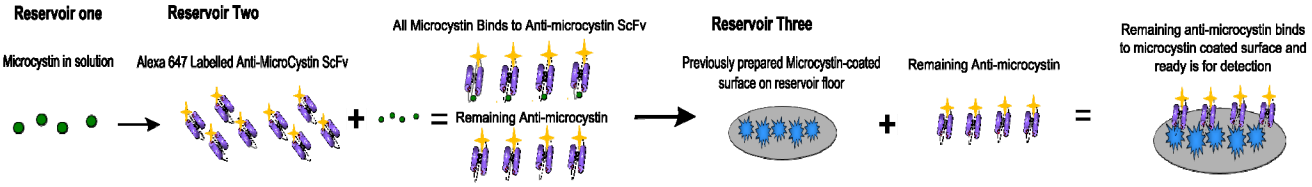

Figure 2: The Microcystin detection assay. A sample consisting of microcystin is loaded into reservoir one. This sample then interacts and binds with excess, free fluorescently-labelled anti-microcystin antibodies in reservoir two. The antibody-antigen pairs and remaining, unbound antibodies progress to reservoir 3, where the microcystin antigen in immobilized on the reservoir floor. The remaining, free fluorescently-labelled anti-microcystin antibodies then bind to this surface where they can be fluorescently detected.

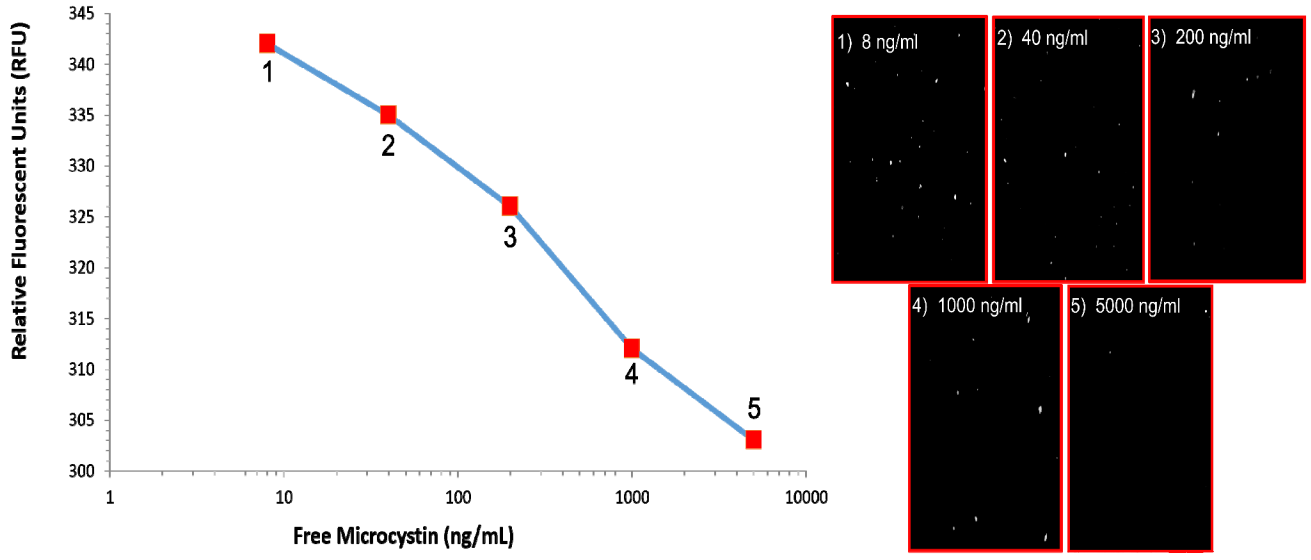

Figure 3: Recorded fluorescence Vs. Microcystin concentration with corresponding fluorescent microscopy images.

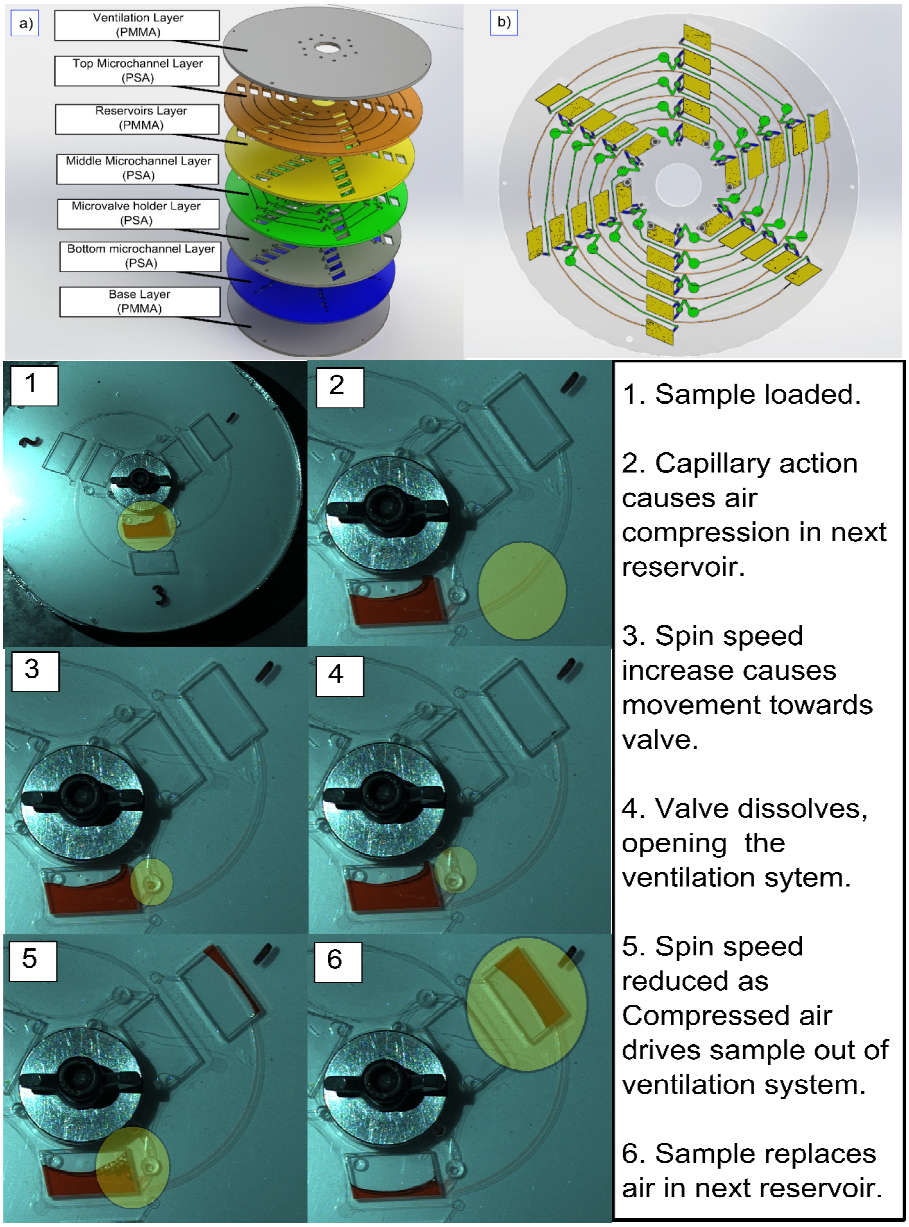

Figure 4: Pneumatic valving system for the automatic ToxiSense disc. Figure 4.a-b illustrates the next generation automation design, with figures 4.1-6 demonstrating the valve automation processes.

Conclusion

Here, we have presented a novel, portable toxin-detection system which has been adapted to detect low concentration levels of microcystin (ng/ml) in samples of water. This cost-effective system can be further modified to allow for autonomous, in-situ detection and real-time monitoring of fresh or brackish water sources.

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