

HIGHLY SCALABLE COMBINATORIAL MIXING OF SAMPLES WITH TARGET-SPECIFIC PRIMERS FOR RAPID PATHOGEN DETECTION ON A CENTRIFUGAL PLATFORM

D.W.Y Chung^a, D.J. Kinahan^a, C. Schoen^b, T. Dreo^c and J. Ducrée^{*a}

^aSchool of Physical Sciences, Dublin City University, IRELAND

^bWageningen University & Research, Wageningen, THE NETHERLANDS

^cNational Institute of Biology, Ljubljana, SLOVENIA

ABSTRACT

In application areas such as crop genotyping, plant diagnostics, pharmaceuticals and forensics, screening a large number of M samples for specific responses to a library of N active agents in a time- and cost-efficient manner is of critical importance. Parameters of interest include response of cells to a specific drug compound, identification of specific genes or plant pathogens in crops using DNA markers and DNA traceability for food safety. The cost of reagents as well as the liquid handling robotics required to perform the enormous number of pipetting steps severely hamper the proliferation of such key technologies into smaller laboratories.

INTRODUCTION

In this paper we present a centrifugal microfluidic platform [1] for automating the combinatorial mixing challenge in a simple instrument. Rather than requiring $2 \times M \times N$ pipetting steps as on bench, loading the disc only requires $M+N$ steps; this scaling implies a decisive reduction of pipetting steps towards large numbers of samples and reagents. The operating principle of the combinatorial disc is adapted from a dissolvable film (DF) based [2] sample aliquoting and metering structure [3]; here a liquid is accurately metered by a sequence of overflow chambers which are individually controlled by a DF burst valve.

This microfluidic platform demonstrates a first step towards highly scalable automation of the liquid handling protocols required for common, combinatorial screening methods on a simple, spindle-motor based instrument. Furthermore, by significantly reducing the number of pipetting steps, by lowering reagent costs through miniaturization as well as by accurate metering and widely eliminating human error in liquid handling, our technology meets the requirements of deployment in decentralized labs.

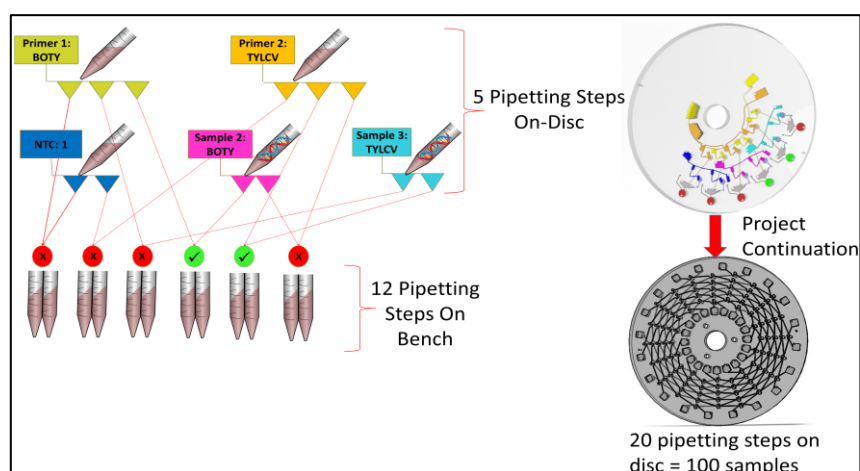


Figure 1: Schematic showing $M \times N$ mixing of samples and reagents with on-disc pipetting steps versus on bench pipetting steps. Rather than requiring 12 pipetting steps, as on bench (i.e. $M \times N \times 2$), loading the disc requires a mere 5 pipetting steps ($M+N$). The reagent and sample storage and directional flow is also highlighted. A progression of the design is shown demonstrating 20 pipetting steps on disc vs 100 pipetting steps on bench

EXPERIMENTAL & RESULTS

As a pilot application, we demonstrate a loop-mediated isothermal amplification (LAMP) of two DNA samples plus an NTC ($M = 3$) for the presence of two pathogens ($N = 2$): Tomato leaf curl virus

(TYLCV) and the fungus *Botrytis Cineria* [4][5]; these pathogens are known to cause tremendous economic loss in tomato plants. Samples and primers are pipetted directly into the disc and first metered to set values of 15 μ l and 10 μ l, respectively. As characterized by absorbance of dyed water [6], we experimentally determined a volume of (9.2 \pm 0.2) μ l for the 10 μ l chamber. Each 15- μ l reaction chamber was pre-filled with 10 μ l of mineral oil to prevent evaporation. For amplification, the cartridge was placed into a pre-heated oven at 70°C for 1 hour. Amplification was confirmed by gel electrophoresis (Fig. 2) where only the samples containing the (known) virus were detected while signals in the negative controls were absent.

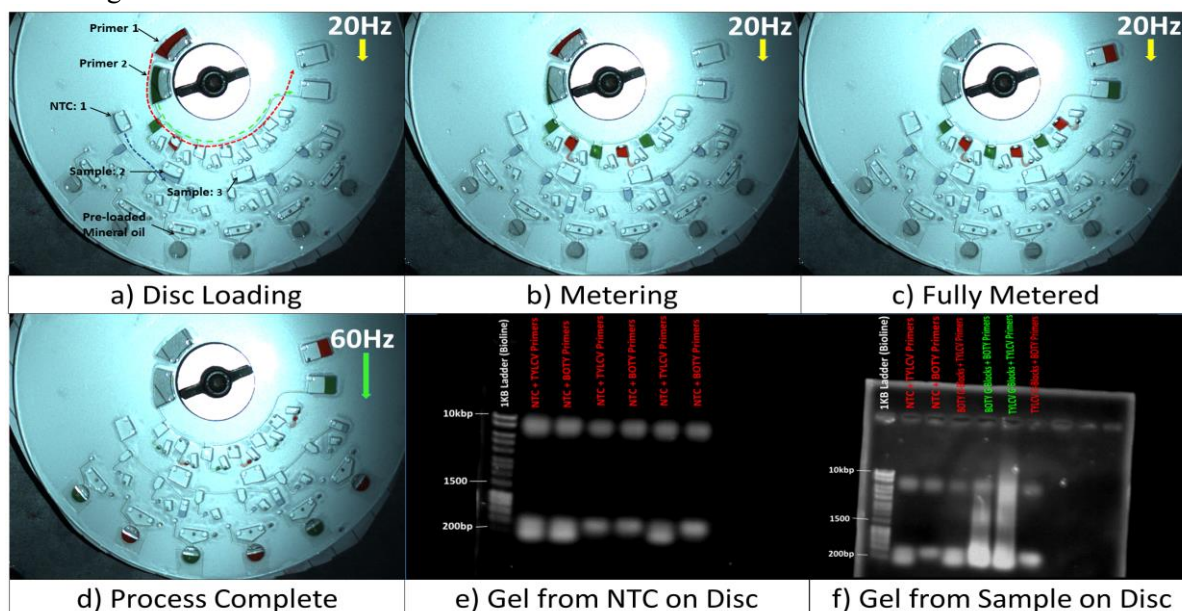


Figure 2: The various coloured food dyes are pre-loaded onto the disc and spun at a frequency of 20Hz. Coloured food dye is used to provide a visual representation of different reagents and samples; red- *BOTY* primer 1, green- *TYLCV* primer 2, Blue- *NTC* sample 1, *BOTY* sample 2 and *TYLCV* sample 3. (a) Each sample (1..M) loaded on the chip is divided into 'N' aliquots while each of the primers (1..N) loaded onto the disc is divided into 'M' aliquots. (b-c) Once metering is completed, the disc spin rate is increased to open the DF burst valves. (d) The liquid elements are then pumped, through a multi-layer architecture, to reservoirs on the periphery of the disc where each individual sample mixes with an individual reagent to create M*N unique reagent combinations. (e-f) Show confirmation of DNA amplification by Gel Electrophoresis.

DISCUSSION

This microfluidic disc was highly optimised to successfully demonstrate M*N microfluidic capabilities and to perform DNA amplification of 2 types of DNA sample – *BOTY* and *TYLCV*. The results show that the microfluidic system is reliable to perform pathogenic DNA analysis with no cross contamination of DNA samples during the process. 2*3 combinatorial mixing was achieved.

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CONTACT

*Jens Ducreé, jens.ducree@dcu.ie , +353-1-700-5377