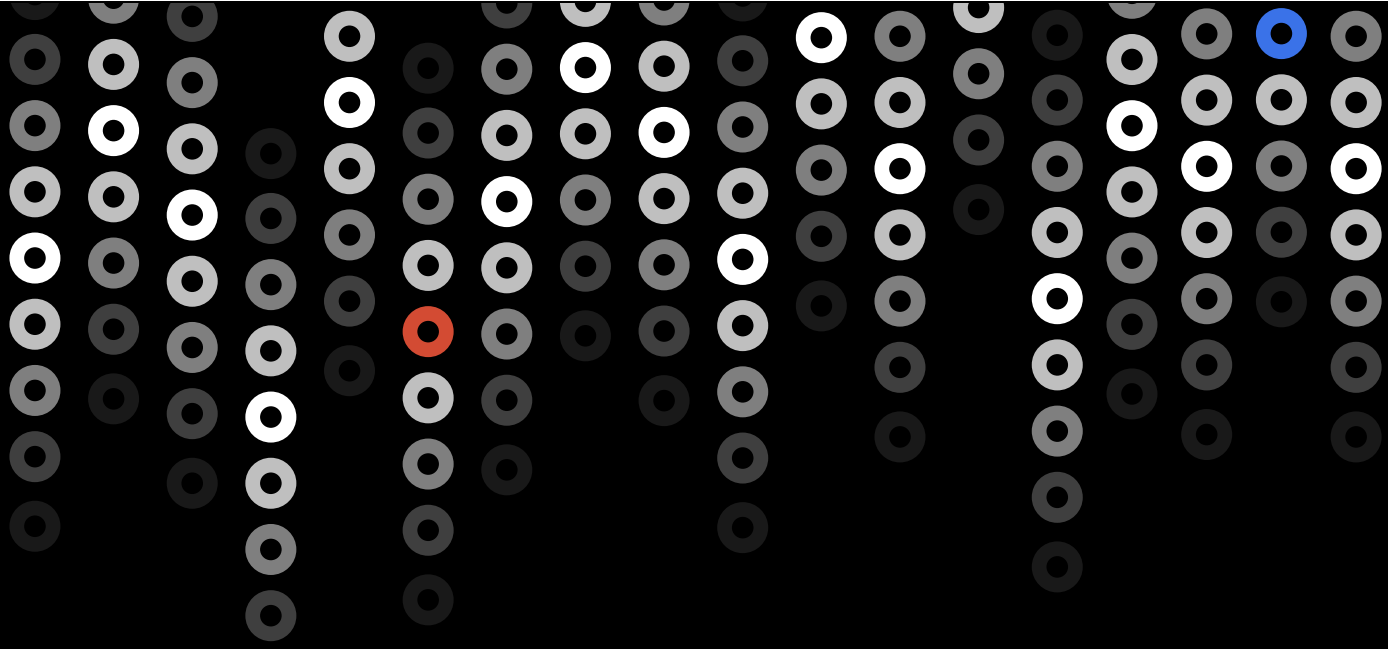


YOUR GROUND CONTROL IN HIGH-THROUGHPUT BIOLOGY

TECHNICAL NOTE

DG-TN0002 – Rev A – Technical Note

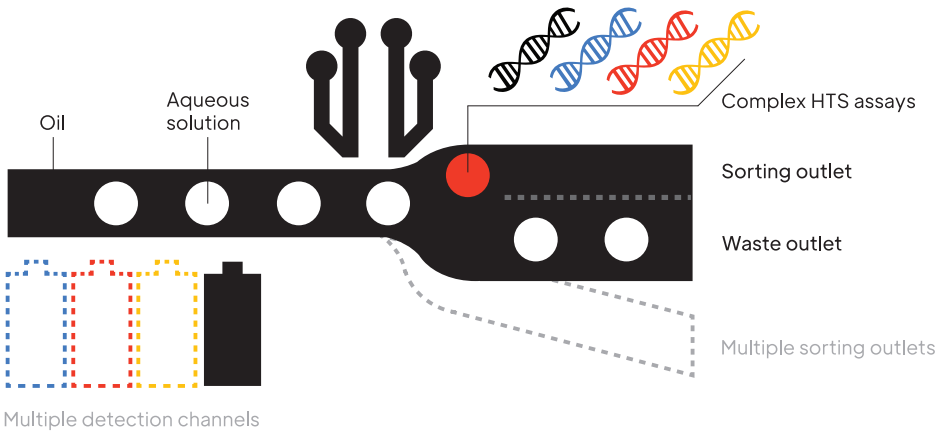
HIGH-THROUGHPUT SCREENING POWERED BY MICROFLUIDICS-BASED DROPLET SORTING

Droplet Genomics

High-throughput screening powered by microfluidics-based droplet sorting

SUMMARY:

- Sorting based on dielectrophoretic actuation is simple and reliable which has made it a widespread droplet selection approach.
- Exciting improvements of fluorescence-activated droplet sorting may broaden the scope of microfluidics-based high-throughput screening experiments.



INTRODUCTION

Miniaturization of screening systems can considerably improve high-throughput screening (HTS) efficiency and reduce costs, enabling the exploration of very large libraries. These opportunities have stimulated the developments in microfluidics that now provide droplet generation, droplet manipulation, and droplet screening capabilities. With the droplet generation rate reaching tens of kHz[1], the speed and efficiency of droplet screening and sorting usually limits the efficiency of the whole microfluidics-based workflow[2].

Several droplet HTS approaches have emerged including labeled and label-free droplet selection methods. The choice of a sorting approach depends on aspects such as intended cell type, assay type and yield, and others. If the HTS assay outcome can be linked to the change of the optical signal, the primary choice of sorting method would be fluorescence-activated droplet sorting (FADS). FADS is the most mature on-chip droplet selection technique with the following advantages:

- sensitive detection, e.g., 2.5 nM of fluorescein in 2 pL droplets[3];
- allows to sort a broad range of droplet volumes – from 20 fL to 10 nL[4];
- sorting rates might be as high as 30 kHz, which is comparable to the speed of fluorescence-

activated cell sorting (FACS)[5];
- the high-speed camera allows visualizing each droplet sorting event.

Active sorting can be achieved by several actuation methods, including acoustic, magnetic, pneumatic, thermal, and electric[6]. Among them, dielectrophoretic actuation is the most simple and reliable means of droplet selection. Moreover, it is driven by electronics that provide high spatial and temporal controllability. These properties enabled the democratization of dielectrophoretic sorting.

A typical dielectrophoretic sorting chip contains a sorting junction linked to two asymmetric channels. By default, droplets flow into a wider waste channel due to lower hydrodynamic resistance. Aqueous droplets have a higher dielectric constant compared to the surrounding continuous phase, thus when an electric field is applied, droplets are pulled into a hydrodynamically less favorable sorting channel.

VARIATIONS OF FADS

A notable limitation of a traditional FADS design is the binary nature of sorting. Multiparametric screening would open up opportunities to sort samples according to the strength of phenotype which would result in obtaining truly quantitative and mechanistic data. This limitation is being tackled by the introduction of more complex microfluidic chip designs.

Multiplexed sorting

Sorting droplets into multiple outlets has recently provoked enthusiasm since it enables the extraction of more than one droplet population from a heterogeneous mixture. Multiplexed sorting can be performed either by sequential integration of multiple binary sorters[7] or by tuning the strength of the applied force[4]. The first option has a severely limited throughput of only a few droplets per second. The throughput of the second approach might be up to 200 Hz.

A single dielectrophoretic sorting device with multiple outlet channels can be used when droplet deflection is controlled by the variation of amplitudes of dielectrophoretic forces. Caen et al. have demonstrated the sorting of 5 populations of droplets simultaneously with a success rate higher than 98.4%[4].

Multiplexed sorting combined with downstream analyses, such as next-generation sequencing, could bring more informativeness to HTS assays by empowering to interrogate, for example, enzyme variants of different activity in directed evolution studies.

Improved detection

To enable multiparametric screening, more complex detection modes can be designed. Ma et al. have proposed a dual-fluorescence detection/sorting microfluidic device to screen for two different chemical reactions simultaneously[8]. The authors were able to select esterase mutants with both high enzymatic activity and high enantioselectivity with this approach.

Apart from the use of different fluorogenic substrates, other characteristics of fluorescence have been employed to trigger sorting events, such as fluorescence lifetime. Hasan et al. developed a setup able to distinguish droplets containing pyronine and fluorescein with accuracy of more than 92%, although both fluorophores were excited at the same wavelength[9]. The advancement of fluorescence lifetime-based sorting might help to expand the scope of FADS even more.

CONSIDERATIONS

How important is the monodispersity of droplets for the sorting procedure? Droplet size uniformity is a critical parameter for highly quantitative assays for several reasons. First, it guarantees consistent signal intensities that allow setting a reliable sorting threshold. Moreover, even size ensures predictable deflection of each of the droplet populations at a given voltage, especially in multiplexed sorting experiments. Droplet Genomics has developed the Onyx system to generate monodisperse emulsions for a wide variety of experimental needs.

Is on-chip droplet sorting compatible with oil-in-water or water-in-oil-in-water double emulsions? FADS is tailored to work with water-in-oil emulsions, although attempts to study the responsiveness of double emulsion droplets to dielectrophoretic force in microfluidic channels have been reported[10]. We recommend considering FACS or droplet sorting by pneumatic control when dealing with emulsion having an aqueous continuous phase.

DROPLET SORTING WITH DROPLET GENOMICS STYX SYSTEM

HTS and droplet sorting assays might come in many shapes and serve numerous purposes. Droplet Genomics Styx system eliminates the technical complexity of FADS setup while retaining maximum design flexibility to enable any high-throughput functional screening campaign.

Styx is equipped with up to 4 lasers, 4 independent fluorescence detection channels, and a high-voltage pulse generator. Exceptional real-time control of the progress of droplet cytometry or sorting run is enabled by dual high-speed microscopy setup (two areas of the sorting chip

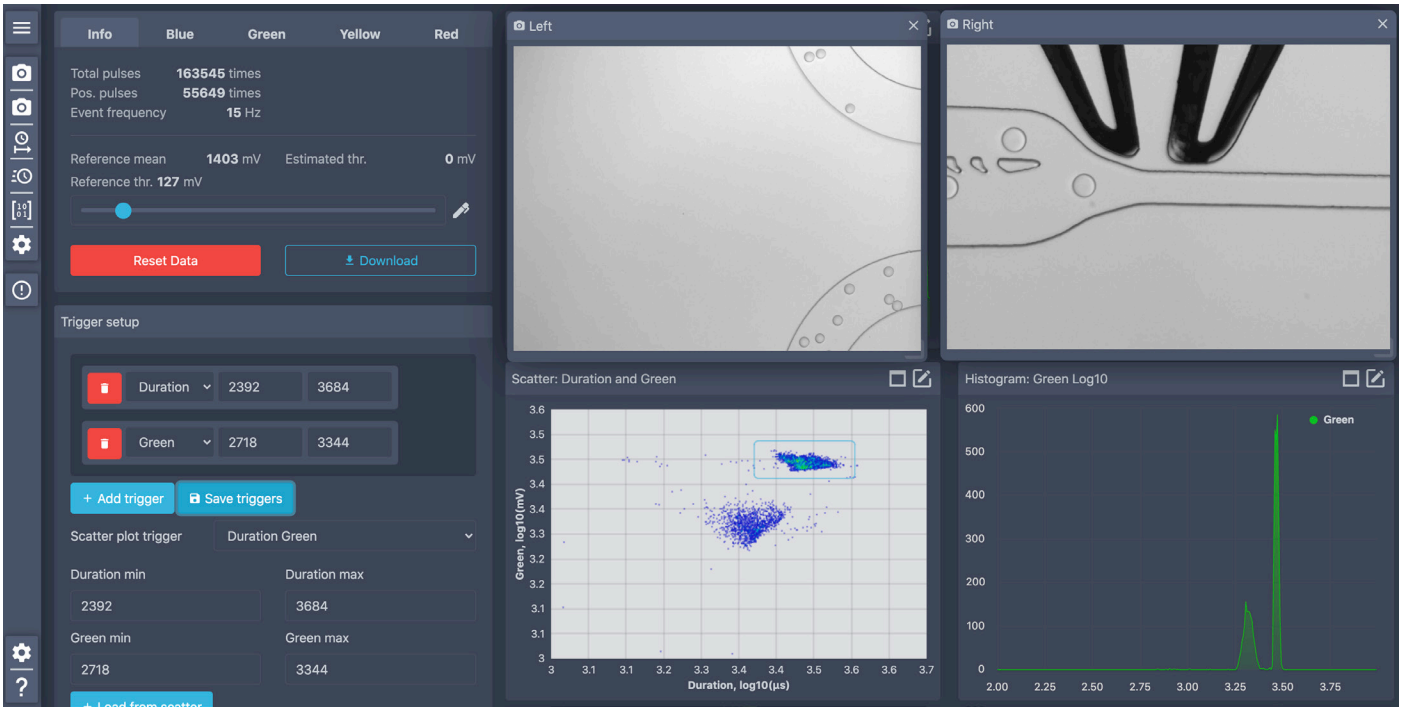


Figure 1. Dual high-speed microscopy setup and real-time fluorescence data analysis allow to monitor the run and finetune sorting parameters.

are visible) and remote control capability. Real-time fluorescence analysis streamlines droplet cytometry workflows and allows tight control of sorting parameters (Figure 1).

The complexity and informativeness of HTS largely depend on the design of a microfluidic chip. Figure 2 shows a typical FADS experiment with a binary sorting device. Nevertheless, Styx is not posing any limits for assay design as the instrument is compatible with any commercially available or custom-made elastomeric chips.

CONCLUSIONS

- FADS is a droplet sorting method of choice if the HTS assay can produce a change of fluorescence signal.
- The efforts to increase the informativeness of FADS include the development of microfluidic devices compatible with multiplexed sorting and employing more complex detection modes.
- Droplet Genomics Styx system supports any type of FADS-based high-throughput screening.

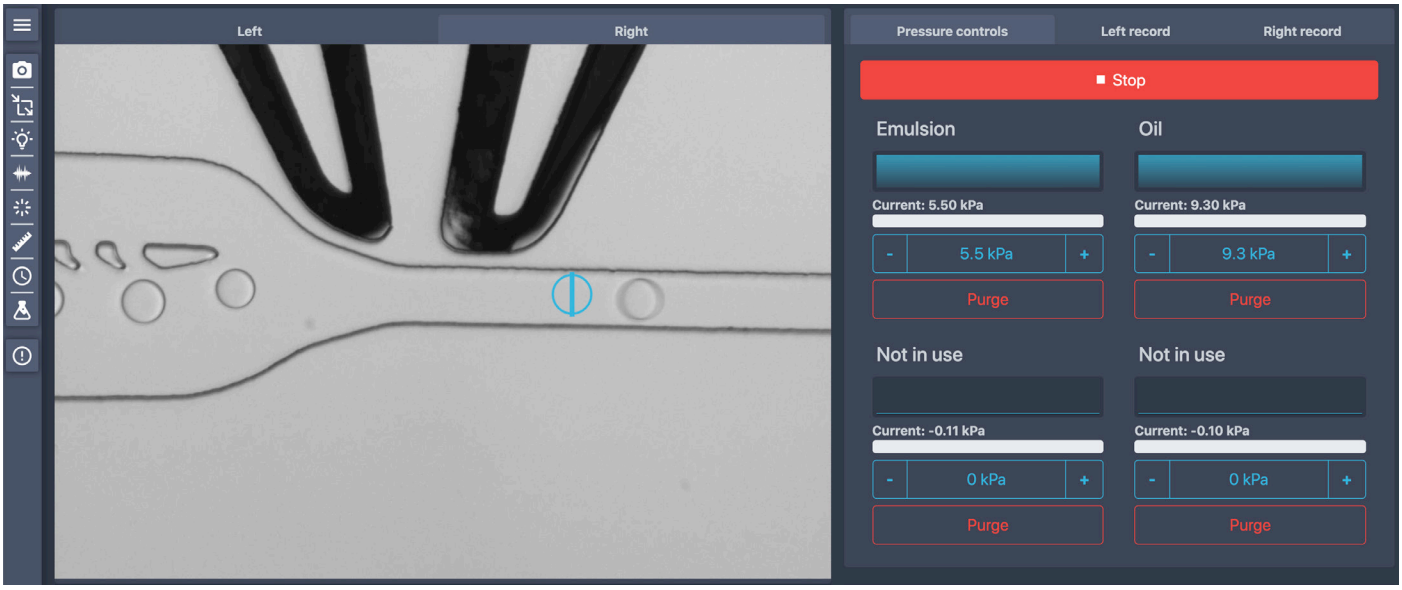


Figure 2. Droplet sorting with the Styx system.

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ORDERING INFORMATION

Product	Quantity	Cat. No.
Instruments		
Onyx	1 instrument	DG-ONYX
Styx	1 instrument	DG-STYX
Kits		
Styx Microfluidic Tubing Kit	5 runs	DG-KIT-STX-MT
Droplet Selection Kit	5 runs	DG-KIT-SLC
Reagents & Consumables		
Droplet Stabilization Oil	15 mL	DG-DSO-15
Spacing Oil	15 mL	DG-SPO-15
Sample Loading Oil	15 mL	DG-SLO-15
Emulsion Breaker	1 mL	DG-EB-1
Microfluidic Tubing	30 m (OD 0.76 mm)	DG-MT0.3-30
	30 m (OD 1.07 mm)	DG-MT0.56-30
Droplet Selection Device	1 chip	DG-STX-SRT
Custom Microfluidic Chips	1 chip	Please inquire

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For technical assistance, contact
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