

# DIGITAL CHROMATOGRAPHY AND THE FORMATION OF HETEROGENEOUS DROPLET LIBRARIES USING MICROFRACTIONATION IN DROPLETS ( $\mu$ FD)

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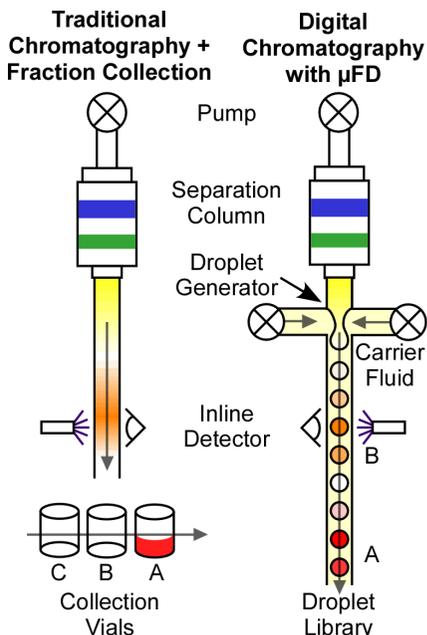
## ABSTRACT

Generating chemical libraries in droplet form is vital to the adoption of microdroplet systems in high-throughput screening (HTS). This paper presents microfractionation in droplets ( $\mu$ FD), a method for generating heterogeneous droplet libraries using standard separation processes. In  $\mu$ FD, compounds eluting from a separation column are permanently encapsulated into droplets by a droplet generator placed immediately after the column. A prototype, comprised of a C18 cartridge and a cross-junction droplet generator, creates a library of organic dyes. Cascaded fiberoptic absorbance detectors generate “digital” chromatograms which report on each droplet’s size, frequency, and composition. This technique can be adapted to many separation processes to form a variety of droplet libraries.

**Keywords:** droplet libraries, fractionation, high throughput screening

## INTRODUCTION

Droplets in an immiscible carrier fluid can be used as isolated reaction containers for HTS, offering dramatically higher throughput and lower reagent costs compared to the traditional microplate format [1]. It has been shown that droplets with  $\mu$ L-fL volumes can be metered, mixed, split, and optically analyzed at rates  $> 1$  KHz [1-3]. However, a fundamental capability, which still remains unsolved, is the ability to create a library of droplets containing the screening compounds. Zheng [4] and Damean [5] generated droplets containing titrations of 2-3 reagents, and used them for screening protein crystallization conditions and enzymatic activity. Many others have developed techniques for generating homogeneous droplets [1-2]. However, to date, there is still no method for generating libraries of  $n$  diverse compounds, and the inability to do so limits the adoption of microdroplet systems in HTS. To address this issue,

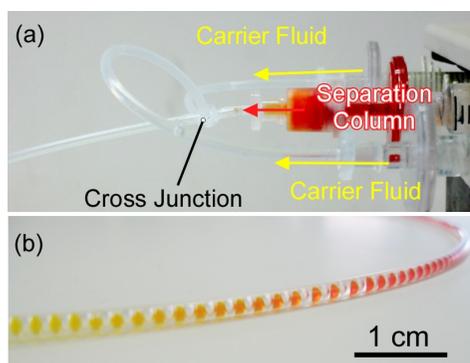


**Fig. 1:** Comparison of traditional fraction collection (left) and microfractionation in droplets (right).

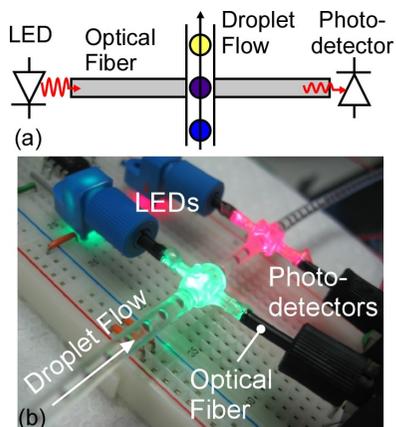
this paper introduces microfractionation in droplets ( $\mu$ FD), a technique which can generate heterogeneous droplet libraries by coupling separation processes with droplet generators (Fig. 1).

## EXPERIMENTAL SETUP

The prototype  $\mu$ FD system consists of three components: a C18 sep-pak cartridge, a droplet generator, and two fiber-optic absorbance detectors. The C18 cartridge separates a mixture of dyes (FD&C Red No. 40, Blue No. 1, and Yellow No. 5) using 99% methanol as the eluent. As the separated compounds exit the column, they are immediately encapsulated into droplets by the droplet generator (Fig. 2a). Similar to a flow-focusing generator [2], the cross junction forms monodisperse droplets in an immiscible carrier fluid due to orthogonal shear forces which break off droplets in a repeatable manner. Once encapsulated, the droplets are chemically isolated from one another by the immiscible carrier fluid. Two inline optical detectors, each consisting of a fiber-optic LED and a phototransistor (Fig. 3), provide absorbance measurements at 660 and 530 nm wavelengths. The photodetector signals are recorded using a multi-channel data acquisition card, and post-processed using MATLAB.



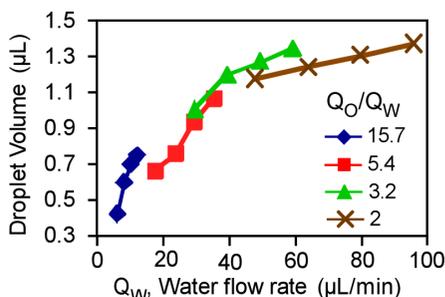
**Fig 2:** (a) Experimental setup, showing a C18 cartridge loaded with red and yellow dyes, and a plastic cross-junction droplet generator. (b) Droplet library formed in a 1.5 mm ID tube.



**Fig 3:** Schematic (a) and experimental setup (b) of a dual wavelength, fiber-optic absorbance detector.

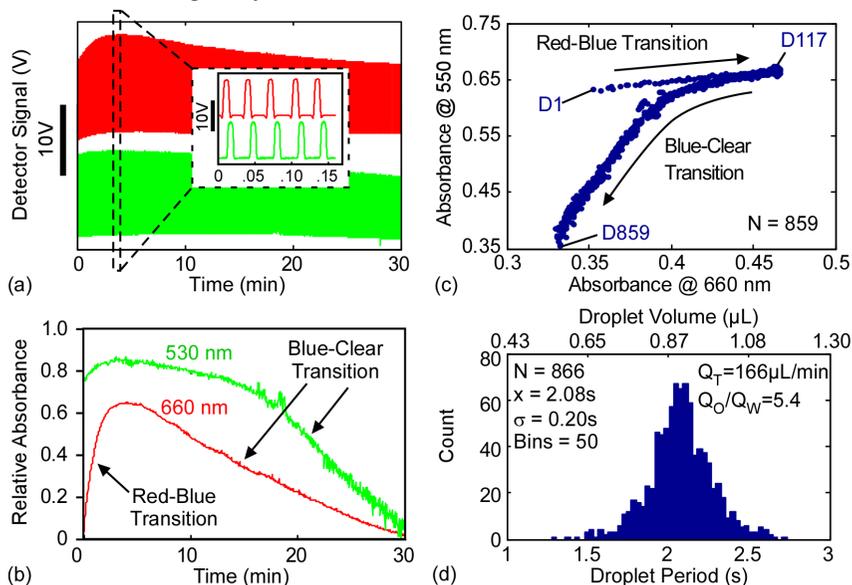
## RESULTS AND DISCUSSION

A droplet library of yellow and red dyes generated using the prototype system is shown in Fig. 2b. Separation efficiencies, as well as the purity of the resulting fractions, can be improved by using high performance liquid chromatography (HPLC). The size of the droplet fractions can be tuned by adjusting the diameter of the droplet generator [2] or the relative flow rates (Fig. 4). As the drop-



**Fig 4:** Droplet volume vs. the flow rates of water and the carrier fluid.

let fractions pass by the optical detectors, they generate peaks in the absorbance signal which represent a “digital sampling” of the chromatogram (Fig. 5a). The system records each peak’s amplitude and width, which correspond to the droplet’s concentration and size, respectively. Envelope detection can be used to reconstruct the traditional “analog” chromatogram (Fig. 5b). In addition, the discrete absorbance data at multiple wavelengths allows droplet populations to be plotted on a 2-D scatter plot, similar to cell cytometry (Fig. 5c). Such multi-dimensional analysis enables the  $\mu$ FD system to “fingerprint” the composition of each droplet in the library before it is used in a screening assay.



**Fig. 5:** Digital chromatography of red and blue dyes using  $\mu$ FD and dual detectors. (a) Raw data. (b) After envelope detection, showing absorbance at 530 nm and 660 nm. (c) 2-D scatter map for identifying droplet composition. (d) Histogram of droplet volume and period.

## CONCLUSIONS

The  $\mu$ FD prototype shown in this paper generates a library of coloring dyes using a C18 cartridge as the separation method. In principle, however,  $\mu$ FD can be coupled to any separation process. Therefore, it can create a diversity of droplet libraries, including DNA, proteins, and small molecules. Such libraries are useful for HTS in genomics, proteomics, and drug discovery.

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