

## Supplementary Information for

### A Digital Microfluidic Method for Dried Blood Spot Analysis

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## **Fabrication Materials**

Concentrated sulfuric acid and hydrogen peroxide (30%) were from Fisher Scientific (Ottawa, ON), Parylene C dimer was from Specialty Coating Systems (Indianapolis, IN) and Teflon-AF was from DuPont (Wilmington, DE). Shipley S1811 photoresist and MF321 developer were from Rohm and Haas (Marlborough, MA), AZ300T photoresist stripper was from AZ Electronic Materials (Somerville, NJ), solid chromium was from Kurt J. Lesker Canada (Toronto, ON), CR-4 chromium etchant was from Cyantek (Fremont, CA), hexamethyldisilazane (HMDS) was from Shin-Etsu MicroSi (Phoenix, AZ), and Fluorinert FC-40 was from Sigma-Aldrich Canada (Oakville, ON). Piranha solution was prepared as a 3/1 v/v mixture of sulfuric acid/hydrogen peroxide. Dicing tape (medium tack) was purchased from Semiconductor Equipment Corp. (Moorpark, CA).

## **Digital Microfluidic Device Fabrication and Operation**

Digital microfluidic devices were fabricated in the University of Toronto Emerging Communications Technology Institute (ECTI) cleanroom facility, using a transparent photomask printed at Norwood Graphics (Toronto, ON). Device bottom plates bearing patterned chromium electrodes were formed by photolithography and etching as described previously,<sup>1</sup> and were coated with 2.5  $\mu\text{m}$  of Parylene-C and 50 nm of Teflon-AF. Parylene-C was applied using a vapor deposition instrument (Specialty Coating Systems), and Teflon-AF was spin-coated (1% wt/wt in Fluorinert FC-40, 2000 rpm, 60 s) followed by post-baking on a hot-plate (160 °C, 10 min). The polymer coatings were removed from contact pads by gentle scraping with a scalpel to facilitate electrical contact for droplet actuation. In addition to patterned bottom plates, unpatterned indium tin oxide (ITO) coated glass substrates (Delta Technologies Ltd, Stillwater, MN) were coated with Teflon-AF (50 nm, as above) to serve as device top plates.

The bottom plate device design used for off-line analysis featured an array of eighty-eight actuation electrodes ( $2.2 \times 2.2$  mm ea.) connected to ten reservoir electrodes ( $5 \times 5$  mm ea.), with inter-electrode gaps of  $40 \mu\text{m}$ . For method 1 (Fig. 2a in the main text), devices were assembled with an unpatterned ITO-glass top plate and a patterned bottom plate separated by a spacer formed from four pieces of double-sided tape (total spacer thickness  $360 \mu\text{m}$ ). For method 2 (Fig. 2b in the main text), the two plates were separated by six pieces of double-sided tape (total spacer thickness  $540 \mu\text{m}$ ). To actuate droplets, driving potentials ( $70\text{--}100 V_{\text{RMS}}$ ) were generated by amplifying the output of a function generator (Agilent Technologies, Santa Clara, CA) operating at 18 kHz. As described elsewhere,<sup>1,2</sup> droplets were sandwiched between the two plates and actuated by applying driving potentials between the top electrode (ground) and sequential electrodes on the bottom plate via the exposed contact pads. Droplet actuation was monitored and recorded by a CCD camera mounted on a lens.

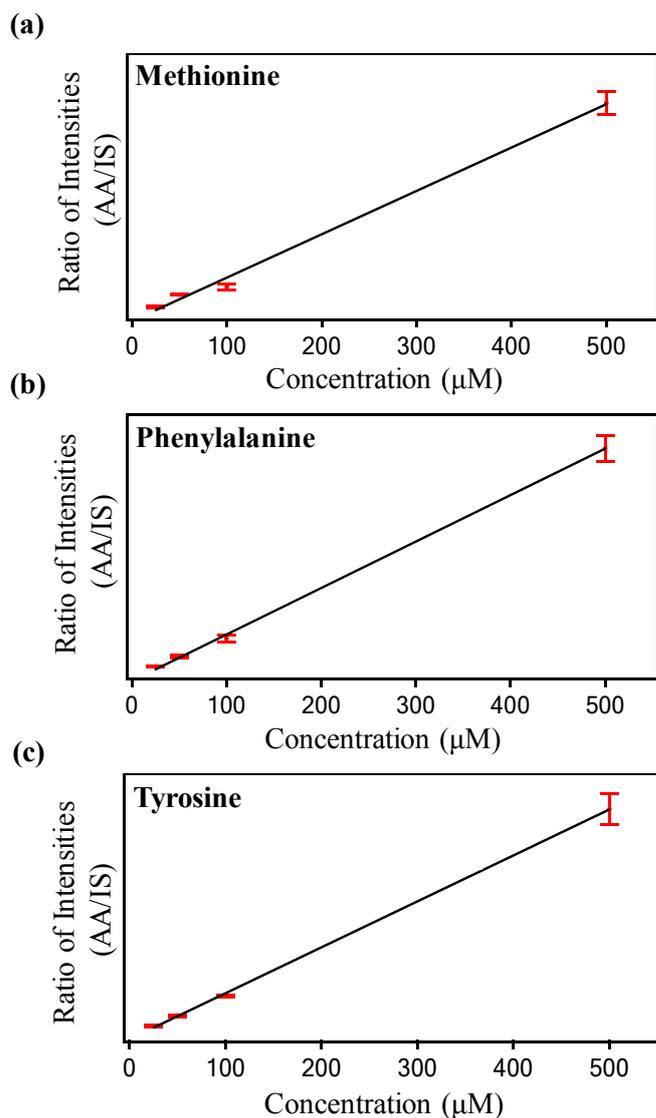
### Hybrid Device Fabrication and Operation

Hybrid DMF-microchannel devices used for in-line analysis were fabricated in four steps. First, a DMF bottom substrate (layer 2 in Figure S3) was fabricated as described above but with no parylene or Teflon-AF coating. The design, shown in Figure 5a in the main text, was similar to that of the DMF-only devices, but with fewer electrodes – 2 rows of 9 actuation electrodes ( $2.2 \times 2.2$  mm) and 3 reservoir electrodes ( $5 \times 5$  mm). The substrates were modified by drilling a hole ( $\sim 2$  mm diameter) through the substrate using a micro drill-press before photolithography. After patterning the electrodes, the opposite side of this substrate (i.e., layer 2 in Figure S3) was coated with  $7 \mu\text{m}$  of Parylene-C. Second, a glass substrate bearing a microchannel nanoelectrospray tip (layer 3 in Figure S3) formed in Parylene was fabricated using methods similar to those described previously,<sup>3</sup> but with the following modifications: the substrate was 25

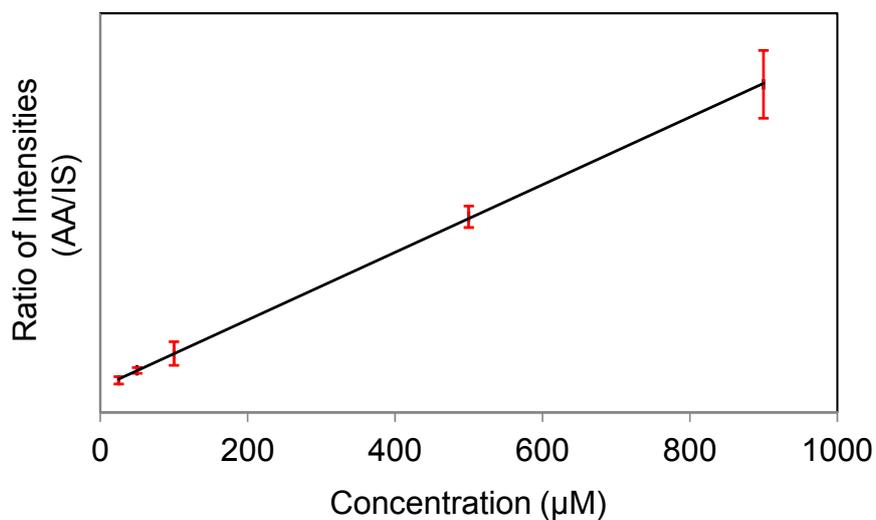
x 55 mm x 1 mm, it was coated with 37 grams of Parylene-C, and the microchannel pattern formed in the parylene was 25  $\mu\text{m}$  wide x 5 mm long. Third, the channel-side of the parylene/microchannel substrate (i.e., layer 3 in Figure S3) was brought into contact with the non-electrode (bare parylene-coated) side of the DMF electrode substrate (i.e., layer 2 in Figure S3), placed under pressure in a precision vise ( $\sim 20$  MPa), and thermally bonded in a vacuum oven ( $200^\circ\text{C}$ , 24 h). After cooling, the electrode-side of the bonded substrate (i.e., layers 2-3 in Figure S3) was coated with 2  $\mu\text{m}$  of parylene (as above) followed by spin-coating 50 nm of Teflon-AF (as above) with a small piece of dicing tape covering the drilled hole. The tape was removed before post-baking on a hot plate ( $160^\circ\text{C}$ , 10 min). Fourth, an unpatterned glass/ITO/Teflon-AF top plate formed as above (layer 1 in Figure S3) was assembled with the thermally bonded substrate (layers 2-3 in Figure S3) from spacers formed from four pieces of double-sided tape.

## References

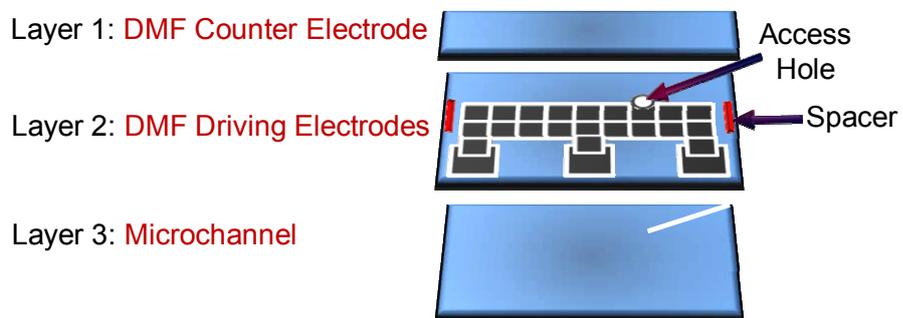
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3. S. L. S. Freire, H. Yang and A. R. Wheeler, *Electrophoresis*, 2008, **29**, 1836-1843.



**Figure S1.** Calibration curves generated by DMF method 1 for quantification of (a) methionine (Met), (b) phenylalanine (Phe), and (c) tyrosine (Tyr). Data were generated by plotting the intensity ratios of the daughter ions of each amino acid (AA) relative to their deuterated internal standard (IS) (i.e., d3-Met, d5-Phe, d4-Tyr, respectively) as a function of AA concentration. Each data point represents at least four replicate measurements, and error bars represent  $\pm 1$  S.D. Regression lines were linear with  $R^2 > 0.996$  for each analyte. Detection limits were calculated as the absolute amount of AA (from each curve) correlating with the average signal measured on a blank sample plus three times the S.D. of that measurement.



**Figure S2.** Calibration curve generated by DMF method 2 for quantification of phenylalanine (Phe) in patient dried blood spots. Data were generated by plotting the intensity ratios of the daughter ions of Phe relative to their deuterated internal standard (IS) as a function of Phe concentration. Each data point represents at least four replicate measurements, and error bars represent  $\pm 1$  S.D. The regression line was linear with  $R^2 > 0.999$ .



**Figure S3.** Exploded view schematic of the hybrid DMF-microchannel device used for method 3 for in-line analysis by mass spectrometry.