Supplementary Information for

A Direct Interface between Digital Microfluidics and High Performance Liquid Chromatography – Mass Spectrometry

Chang Liu,[†,‡,‖] Kihwan Choi,[†,Δ,#,‖] Yang Kang,[‡] Jihye Kim,[†] Christian Fobel,[†,Δ] Brendon Seale[†]

J. Larry Campbell,[‡] Thomas R. Covey,[‡] and Aaron, R. Wheeler[,†,§,Δ]*

[†]Department of Chemistry, University of Toronto, 80 St. George Street, Toronto, Ontario M5S 3H6, Canada

[‡]SCIEX, 71 Four Valley Drive, Concord, Ontario, L4K 4V8, Canada

[§]Institute of Biomaterials and Biomedical Engineering, University of Toronto, 164 College Street, Toronto, Ontario, M5S 3G9, Canada

[Δ]Donnelly Centre for Cellular and Biomolecular Research, 160 College Street, Toronto, Ontario, M5S 3E1, Canada

#Current address: Division of Metrology for Quality of Life, Korea Research Institute of Standards and Science, Yuseong-gu, Daejeon 305-340, Korea

[‖]C.L. and K.C. contributed equally to this work

*E-mail: aaron.wheeler@utoronto.ca

Fluorescence Experiments. Sodium fluorescein and rhodamine 6G were obtained from Sigma-Aldrich (Oakville, Ontario, Canada). In one set of experiments, a 5-μL aqueous droplet containing 20 μM fluorescein was manually loaded into the top-plate access hole. A 2-μL methanolic droplet containing 20 μM rhodamine 6G was dispensed onto the array of electrodes and driven to the access hole to merge with the pre-loaded fluorescein. The combined droplet (partly in the access hole, and partly on the driving electrodes) was actuated to move back and forth on the electrodes adjacent to the access hole for 30 s, after which a unit droplet (~0.85 μL) was split from the mixture and moved away from the hole for analysis in a plate reader (see below). In another set of experiments, an aqueous droplet (5 μL, containing 20 μM fluorescein) and a methanolic droplet (2 μL, containing 20 μM rhodamine 6G) were dispensed onto the array of electrodes of a device without an access hole. The droplets were merged and mixed for 30 s by moving the merged droplet in a square pattern.[30,31] In both experiments, at the completion of the droplet manipulation routines, the devices were affixed to an empty 96-well Stripwell plate, and inserted into a well-plate reader (Pherastar, BMG LabTech, Cary, NC) for fluorescence measurements ($\lambda_{ex}/\lambda_{em}$= 490/520 nm for fluorescein, $\lambda_{ex}/\lambda_{em}$ = 520/580 nm for rhodamine 6G, focal height = 10.6 mm, gain for fluorescein = 100, gain for rhodamine 6G = 500).

Mixing in the "Pre-Wetting" Strategy. As described in the main text, one method developed for injection of methanolic droplets into the autosampler is to "pre-wet" the access hole with a droplet of water. To test the ability to mix the two fluids in this format, a series of experiments was carried out in which a 2-μL methanol droplet containing rhodamine 6G was driven to an access-hole to merge with a pre-loaded 5-μL droplet of water containing fluorescein. After 30 s of active mixing (i.e., agitating the partially wetted droplet on the electrodes around the access-hole), a unit droplet was split from the mixture and driven to a designated electrode for measurement of fluorescence intensity using a plate reader. For comparison, a "well-mixed" sample was performed without pre-wetting on a conventional device with no access hole – that is, a 5 μL droplet of water containing fluorescein was mixed with a 2-μL droplet of water containing rhodamine 6G was merged and mixed, and then analyzed using the plate

reader. The fluorescence intensity ratio of these two fluorophores for the pre-wetted case (1.00 ± 0.03, 1 standard deviation, n=4) was not significantly different from the result of the control system without pre-wetting (0.96 ± 0.07, 1 standard deviation, n=4). From this, we conclude that the pre-wetting strategy does not interfere with the capacity to mix the solutions prior to injection.