Supplementary Material (ESI) for Lab on a Chip

A simple method for the evaluation of microfluidic architecture using flow quantitation via a multiplexed fluidic resistance measurement

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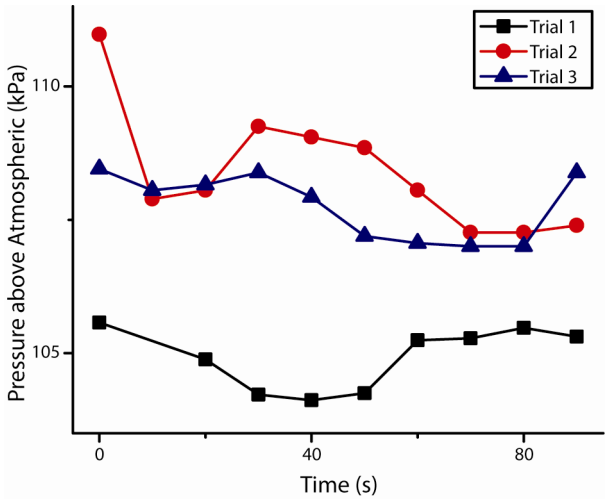
Supplemental Information

Supplemental Table 1. The absorbance measurement method was tested against gravimetric analysis for the same samples for tartrazine (T), sunset yellow (S), fast green (F) and erioglaucine (E). Three orders of magnitude were tested individually and as four dyes combined in the same container. The magnitude of the difference divided by the gravimetric result is shown as an average percentage (n=3).

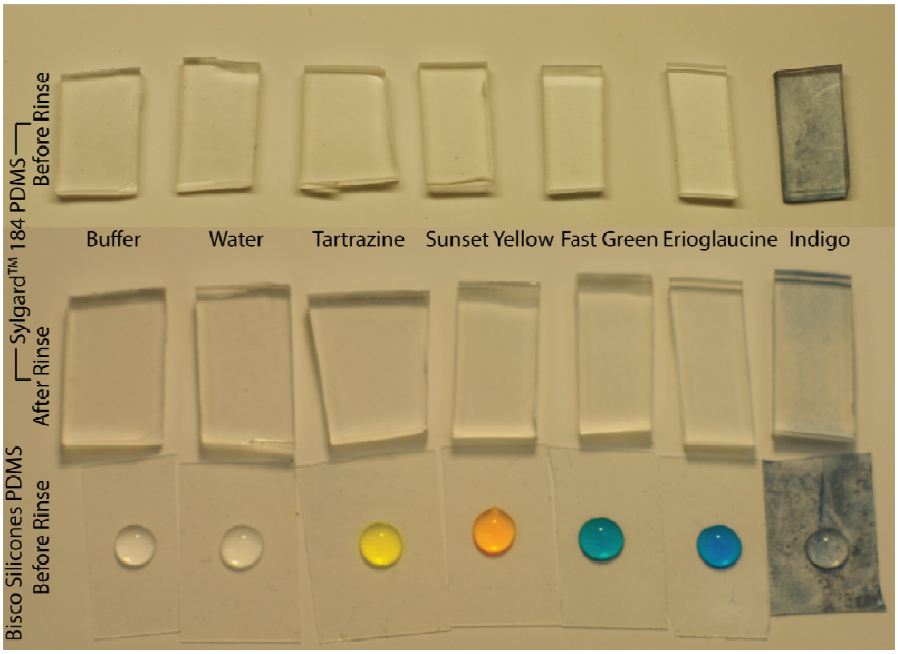
	5 µL			50 µL			500 µL		
Individual Dye Solutions									
Dye	Absorbance volume (µL)	Gravimetric volume (µL)	Difference (%)	Absorbance volume (µL)	Gravimetric volume (µL)	Difference (%)	Absorbance volume (µL)	Gravimetric volume (µL)	Difference (%)
T	5.77 ± 0.15	5.93 ± 0.15	2.78	47.93 ± 1.47	49.83 ± 0.51	3.83	496.0 ± 17.5	489.7 ± 8.1	2.24
S	5.33 ± 0.35	5.72 ± 0.13	6.73	47.90 ± 1.73	50.17 ± 0.46	4.52	494.3 ± 2.5	490.3 ± 7.5	1.50
F	5.53 ± 0.47	5.76 ± 0.34	4.08	47.73 ± 0.91	50.13 ± 0.15	4.79	497.7 ± 15.3	495.3 ± 8.3	3.44
E	5.50 ± 0.35	5.98 ± 0.22	7.98	49.43 ± 0.67	50.07 ± 0.21	1.53	526.7 ± 8.1	491.3 ± 5.7	7.19
4-Component Dye Mixture									
Dye	Absorbance volume (µL)	Gravimetric volume (µL)	Difference (%)	Absorbance volume (µL)	Gravimetric volume (µL)	Difference (%)	Absorbance volume (µL)	Gravimetric volume (µL)	Difference (%)
T	6.07 ± 0.25	5.68 ± 0.11	6.84	50.80 ± 1.83	49.40 ± 0.10	3.10	511.7 ± 17.6	495.7 ± 2.9	3.78
S	5.47 ± 0.06	5.73 ± 0.23	4.53	49.87 ± 1.29	49.00 ± 1.01	2.88	505.0 ± 13.2	496.3 ± 2.3	2.96
F	5.73 ± 0.25	5.89 ± 0.09	2.85	49.60 ± 1.06	49.90 ± 0.30	1.80	473.3 ± 7.6	495.7 ± 2.5	4.50
E	6.10 ± 0.30	6.15 ± 0.16	2.82	51.73 ± 1.22	50.07 ± 0.12	3.33	535.0 ± 13.2	496.0 ± 2.6	7.87



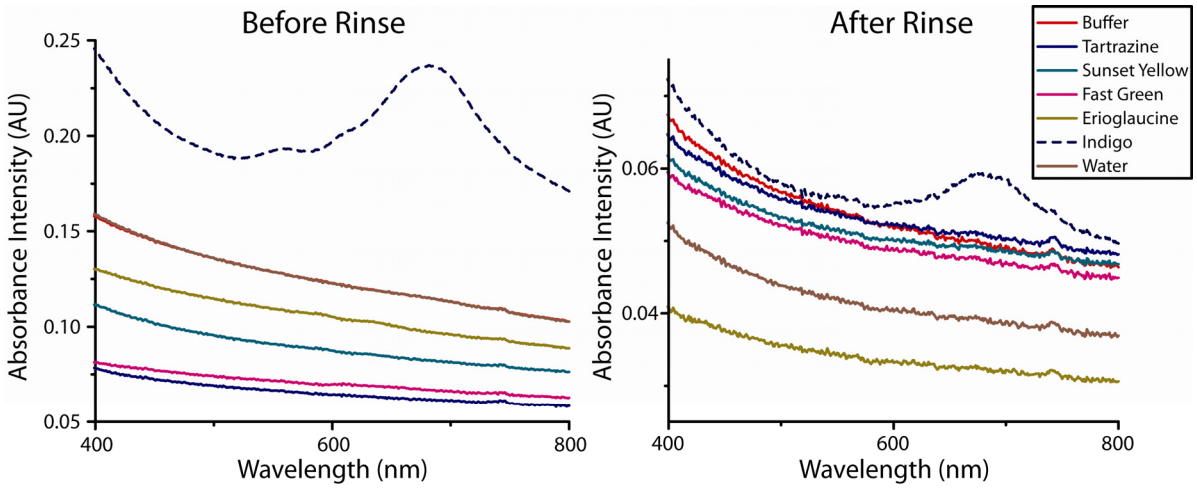
Supplemental Figure 1. Triplicate measurement by MKS pressure transducer of pressure induced by hand depression of syringe plunger to a set point over the course of 90 s. The syringe and fittings are able to reliably apply the pressure over the course of the experiments comparing gravimetric and MFR methods.

Supplemental Table 2. Triplicate measurements by MKS pressure controller of syringe-induced pressures (error is one standard deviation). The positive pressures (with respect to atmospheric pressure) are generated by handheld depression of the syringe plunger to a set displacement for 90 s. The negative pressures are generated by moving the plunger to create a larger volume in the syringe and hold it at that displacement with a spacer (rigid object). Spacer 1 is a blunt end needle and spacer 2 is a metal rod.

	Positive Pressure (by hand)	Negative Pressure (by Spacer 1)		Negative Pressure (by Spacer 2)	
		inital	t=30 min	inital	t=30 min
Trial 1	104.9±0.6 kPa	-48.415 kPa	-48.319 kPa	-68.472 kPa	-68.837 kPa
Trial 2	108.4±1.2 kPa	-48.181 kPa	-48.215 kPa	-68.603 kPa	-69.003 kPa
Trial 3	107.8±0.6 kPa	-48.084 kPa	-48.119 kPa	-69.099 kPa	-69.065 kPa
Average	107±2 kPa	-48.2±0.1 kPa		-68.8±0.3 kPa	



Supplemental Figure 2. Pieces of Sylgard™ 184 and Bisco Silicones HT-6240 PDMS are immersed in dye solutions (same concentrations as experiments) for 65 days. Sylgard samples are rinsed thoroughly with deionized water. No difference in weight was observed by any of the 10 pieces (to the mg) for the duration of the experiment. No partitioning into the bulk substrate of the hydrophilic dyes is observed into the hydrophobic PDMS. A 20-µL drop of each immersion solution is shown on top of the respective piece of Bisco Silicone PDMS in the bottom row.



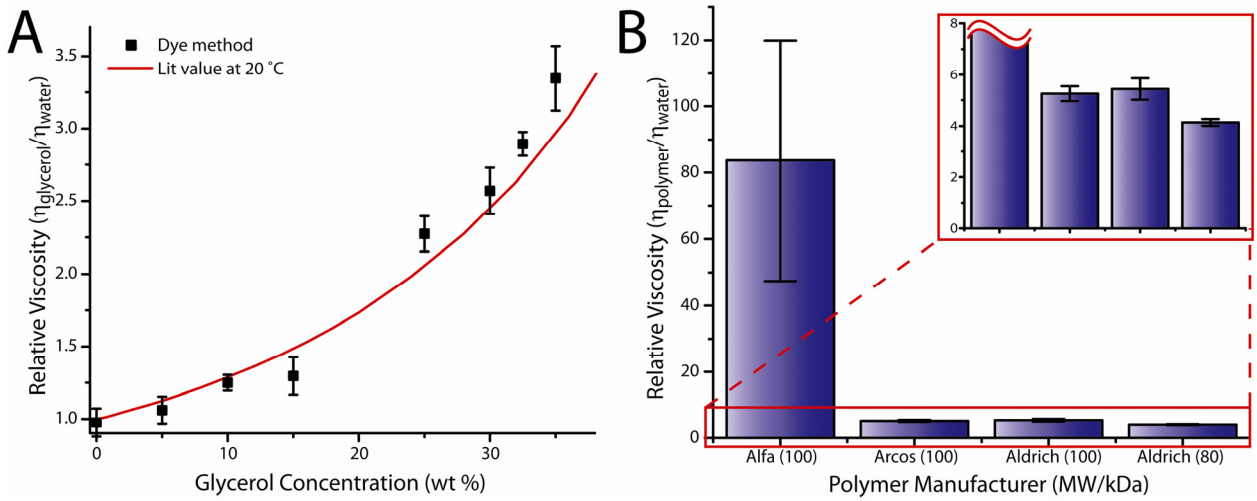
Supplemental Figure 3. Average absorption spectra (n=3) of Sylgard 184 PDMS pieces (pictured in Fig. S-2) after 65 days of immersion in dye solution, buffer, or water. Indigo was the only dye to significantly adsorb onto the surface of the PDMS (broad organic dye peak at 690 nm), and most of the indigo was rinsed off with water. The other pieces had flat spectra similar to water or buffer.

Viscosity Measurement Using MFR

Since fluidic resistance is directly proportional to the viscosity of a solution, the ratio of the fluidic resistances in identical channels provides the relative viscosity of the solution with respect to the viscosity of water. The previously validated 4-channel microdevice was challenged to measure the viscosity of four different aqueous solutions under flow conditions. The relative viscosity measurements of seven aqueous glycerol solutions (5 to 35 wt%) (Fig. S-4a, black squares) closely match the literature values[1] determined for these solutions (Fig. S-4a, red line), with all measurements taken at 20 ± 1 °C. This small difference in temperature may have reduced the accuracy of the measurements, as the viscosity of aqueous solutions is very sensitive to temperature[2]. The data clearly reproduced the expected trend and are accurate to within 0.5 units of relative viscosity (~0.5 cP).

The simultaneous viscosity measurement approach was then used to characterize the viscosity of a polymer employed for capillary gel electrophoretic (CGE) separation of DNA. Hydroxypropyl cellulose (HPC) has been utilized as the sieving matrix for DNA separations with CGE[3], but the viscosity of polymer solutions can vary significantly based on manufacturer, batch and storage conditions, all of which can impact the separation resolution obtained. The viscosity of many of the polymers used for DNA separation, including HPC, decrease with increasing shear stress[4]. This makes the zero shear viscosity (viscosity with no shear applied) less important than the viscosity under the working conditions in CGE polymer introduction[5], in which typically a pressure is applied to the inlet. HPC obtained from three different vendors for evaluation- three had the same molecular weight (MW) (100 kDa) and one a slightly lower MW (80 kDa)- were tested at the same concentration with a pressure of 69 kPa applied to the inlet reservoirs (Fig. S-4b). The channels were previously measured with only dye in them to provide a reference for the relative viscosity measurement with MFR. As would be expected, the 80 kDa polymer had the lowest viscosity relative to water at 4.1±0.1. Two of the 100 kDa polymers had slightly higher relative viscosities of 5.3±0.3 and 5.4±0.4, but one was an order of magnitude higher at 84±36. This polymer proved too viscous for loading into a capillary with a commercial CE instrument. While, in this case, the anomolous viscosity would have been evident upon use of the polymer solution, one can envision senarios where more subtle differences may not be detected but could have adverse effects on separations. While demonstrated here for microchannel charcterization, this method could quickly be incorporated into a capillary-based viscosity measurement in a CE instrument with visible

wavelength absorbance detection in an automated fashion.



Supplemental Figure 4. The ratio of the resistances for water and a solution in identical channels provides the relative viscosity of the solution with respect to water. (a) Aqueous glycerol solutions confirmed the dye generated relative viscosity measurements (black squares) were similar to literature values (red line). (b) The viscosities of four solutions of HPC (3.5 %w/v) from different vendors of 100 kDa and 80 kDa molecular weight were evaluated [calculated average shear rates (1 standard deviation) left to right of 30(10), 560(30), 520(30), 800(10) s^{-1}]. As expected the 80 kDa polymer had the lowest viscosity. Two of the 100 kDa polymers were similar, but one was significantly higher. Error bars denote 1 standard deviation with n=3.

Experimental

Four samples of hydroxypropyl cellulose (HPC) with molecular weights of 100, 100, 100, and 80 kDa were obtained from Sigma-Aldrich, Acros, Alfa Aesar, and Sigma-Aldrich, respectively. 3.5 % w/v solutions of each polymer were prepared in TE buffer, then each polymer solution was diluted 1:1 with one of the 10X dyes. Tartrazine was used in the Sigma-Aldrich HPC (MW=100 kDa), sunset yellow in the Arcos HPC, fast green in the Alfa Aesar HPC, and erioglaucine in the second Sigma Aldrich HPC sample (MW=80 kDa).

A PR4000 power supply and a Type 640 pressure controller from MKS Instruments (Andover, MA) was used to apply prescribed pressures to the inlet reservoirs via a Plexiglas manifold made in-house. The channels of a 4-channel glass-PDMS device were filled with the polymer/dye mixtures for 1 min and 68.9 kPa pressure was applied to the inlet reservoirs for 60 s. The solutions were collected in the single outlet reservoir in triplicate. Samples were diluted to 1 mL with TE buffer and spectroscopic analysis followed. The procedure is repeated with dye solutions to obtain a relative viscosity measurement with respect to water.

To validate on-chip viscosity measurements 0 %, 9.92 %, 19.97 %, 30.02 %, 49.99 %, 60.08 %, 65.01 %, and 69.94 % weight/weight glycerin (Sigma Aldrich, 99%) in TE solutions were made. Each glycerin solution was diluted 1:1 with a stock solution of one of the dyes (tartrazine, sunset yellow, fast green, and erioglaucine) in sets of four solutions (0 % to 30.02 % and 49.99 % to 69.94 %, respectively). First 5X dyes in TE were run through a 4-channel glass-PDMS chip under 34.5 kPa for 30 s to obtain the fluidic resistance of the channels without polymer. The channels were then filled with aqueous glycerin and dye solutions and placed under a 68.9 kPa pressure for 60 s to obtain the resistance of the channels with more viscous solutions.

The MKS pressure controller was used to measure the applied pressure of the syringe and fittings to the channels. Native PDMS pieces were immersed for 65 days in 91.6 µM, 110.1 µM, 172.8 µM,

246.9 μM, and 300 μM TE buffered solutions of tartrazine, sunset yellow, fast green, erioglaucine, and indigo, respectively. Another two pieces were immersed in pure TE buffer and deionized water for the same duration. After 65 days, the pieces were blotted dry and the absorbance of each was measured with a Cary 5E UV-Vis-NIR dual-beam spectrophotometer. The reference beam was left blank. The Sylgard 184 pieces were rinsed thoroughly with deionized water and absorbance measurements were repeated.

References

1 R. C. Weast, ed., *CRC Handbook of Chemistry and Physics*, CRC Press, Inc., Clevland, OH, 1973-1974.

2 R. C. Hardy and R. L. Cottingham, *Journal of Research of the National Bureau of Standards*, 1949, **42**, 573.

3 J. C. Sanders, M. C. Breadmore, Y. C. Kwok, K. M. Horsman and J. P. Landers, *Analytical Chemistry*, 2003, **75**, 986-994.

4 E. Jimenez-Regalado, J. Selb and F. Candau, *Macromolecules*, 2000, **33**, 8720-8730.

5 C. P. Fredlake, D. G. Hert, B. E. Root and A. E. Barron, *Electrophoresis*, 2008, **29**, 4652-4662.