Supporting Information For:

Rapid Prototyping of Multi-Channel Microfluidic Devices for Single-Molecule DNA Curtain Imaging

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1. Supplemental Methods

Device Fabrication

To construct devices, a high-resolution photomask was designed in DraftSight and purchased from CAD/Art Services, Inc. In the photomask for the Y-channel device, the central channel was designed to have a nominal width of 230 μm. A 50 μm dry-film photoresist (DuPont MX5050) was used to generate the PDMS mold master by following the procedure discussed in the experimental section of the manuscript. For the gradient mixer, the central channel was designed to be 1140 μm wide and each of the five individual imaging channels was designed to be 120 μm wide (see Fig. S2). For the gradient mixer device, two layers of MX5050 photoresist were laminated together to give a photomask thickness of ~90 μm. PDMS was de-gassed under vacuum (Yellow Jacket Super Evac 6 pump) for at least 45 min before being poured over photomask masters.

Scanning Electron Microscopy

Scanning electron microscopy (SEM) was used to characterize the reproducibility of PDMS molds cast from dry-film resist master structures. After PDMS molds were cast from the dry-film master structures, both the masters and the PDMS were sputter-coated with a ~10 nm layer of Pt-Pd alloy using a Cressington 208 benchtop sputter coater for imaging with a Zeiss Supra 40 VP scanning electron microscope. To fit within the SEM imaging chamber, the master structures and the associated glass backing had to be scored and cut. This produces small glass shards that are sometimes visible in the SEM images, but are not present in the actual devices. These cut pieces were affixed to aluminum specimen mounts with carbon tape (Ted Pella, Inc.), before being sputter-coated and placed on the SEM stage. To image the PDMS microfluidic channels, PDMS molds were cut with a sharp razor blade before being affixed to aluminum specimen mounts with carbon tape, sputter-coated and placed on the SEM stage. To obtain the depth of the channels, cross-sections of the channels in the PDMS molds were cut and mounted with the channels running perpendicular to the mount. Images were analyzed in ImageJ.

Purification of Human Replication Protein A-Green Fluorescent Protein (RPA-GFP)

A plasmid over-expressing human RPA-GFP-His6 was a generous gift from Dr. Mauro Modesti[1]. For purification, plasmid pIF64 was transformed into Rosetta/pLysS cells (Novagen). A single colony was inoculated into 50 ml of LB with 50 μg/ml carbenicillin and 34 μg/ml chloramphenicol and incubated overnight at 37°C with agitation. The next morning, the overnight pre-culture was diluted 100-fold into 6 L of LB + carbenicillin + chloramphenicol and incubated at 37°C with agitation until OD at 600 nm reached 0.6. Once this OD was reached, the solutions were cooled to 16°C on ice in a cold room. Protein expression was induced with 1 mM IPTG and the culture was incubated at 16°C with agitation for 16-18 h. Cells were harvested by centrifuging for 15 minutes at 5000 x g. The supernatant was discarded and the cell pellet was resuspended in 40 ml PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.47 mM KH_2PO_4

[pH 7.4], 1 mM PMSF). The cells were flash-frozen in liquid nitrogen and stored at -80˚C until needed.

The frozen cell paste corresponding to 3 L of starter culture was thawed in lukewarm water and immediately placed on ice. All subsequent steps were performed at 4˚C. One volume of 2x lysis buffer (1 M NaCl, 40 mM Tris-HCl [pH 7.5], 4 mM β-mercaptoethanol, 10 mM imidazole [pH 8], 20% glycerol, 1 mM PMSF) was added to the cells and resuspended by mixing with a pipette. The lysate was sonicated on ice for a total of 90 s (Fisher Scientific 705 Sonic Dismembrator at 75% amplitude; 15s bursts with 90s rests in between). The lysate was then centrifuged at 35,000 rpm for 35 minutes at 4˚C (Ti-45 rotor in Optima XE ultracentrifuge, Beckman-Coulter). A 5 ml HisTrap FF column was pre-equilibrated with 1x lysis buffer using the ÄKTA FPLC (GE Healthcare). The clarified lysate was injected using a 50 ml SuperLoop (GE) and the column was washed with 50 ml of 1x lysis buffer. Protein was eluted with a gradient to 100% elution buffer (500 mM NaCl, 20 mM Tris-HCl [pH 7.5], 2 mM β-mercaptoethanol, 500 mM imidazole [pH 8], 10% glycerol) over 8 column volumes. The protein sample was then dialyzed against 2 L of buffer R (50 mM KCl, 20 mM Tris-HCl [pH 7.5], 1 mM DTT, 0.5 mM EDTA, 10% glycerol) at 4˚C overnight. The next day the protein was loaded onto a 1 ml HiTrap Heparin HP column pre-equilibrated with buffer R and washed with at least 10 ml buffer R. Protein was eluted with a gradient to 100% buffer RE (500 mM KCl, 20 mM Tris-HCl [pH 7.5], 1 mM DTT, 0.5 mM EDTA, 10% glycerol) over 10 column volumes. RPA-containing fractions were loaded on a Sephacryl S-300 HR (GE Healthcare) column pre-equilibrated with buffer R. The protein was then loaded onto a 1 ml Hitrap Q HP column pr-eequilibrated with buffer R, washed with 15 ml buffer R, and then eluted with a gradient to 100% buffer RE over 10 column volumes. Fractions were analyzed on a 10-12% SDS-PAGE gel. The purest RPA-GFP fractions were pooled and dialyzed against 2 L storage buffer (10 mM Tris-HCl [pH 7.6], 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% glycerol) overnight at 4˚C before being aliquotted and flash-frozen in liquid nitrogen for storage at -80˚C.

Y-channel Device

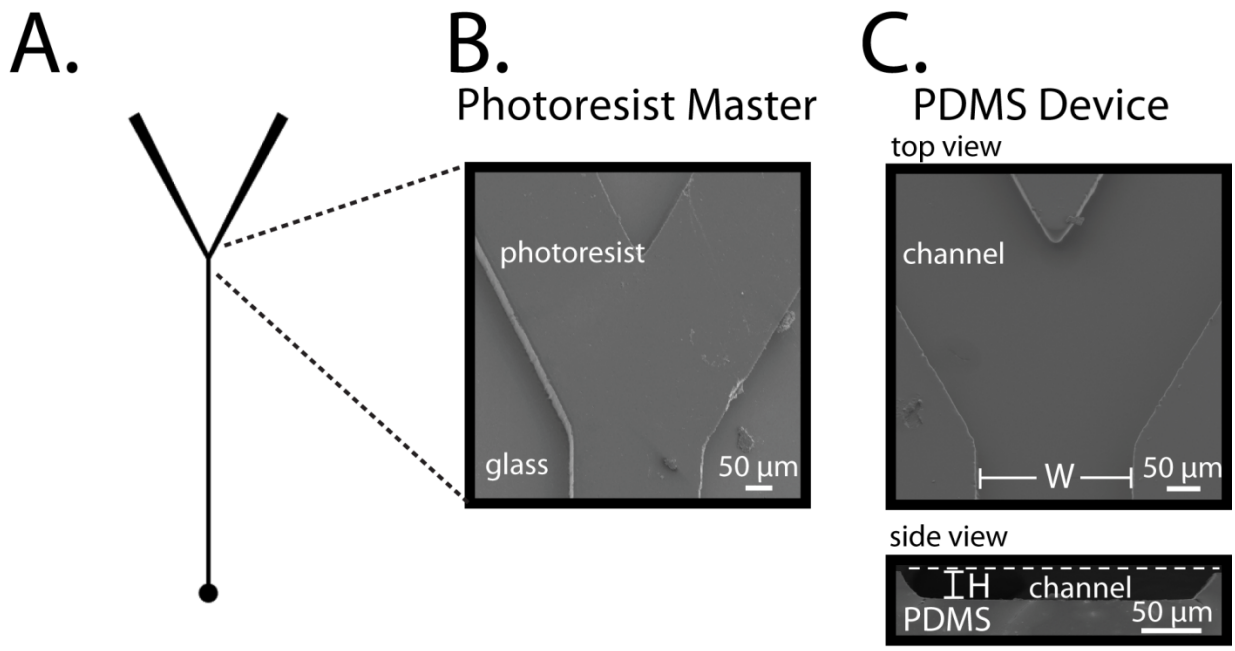

Figure S1. Characterization of Y-channel devices. (A) Schematic illustration of the Y-channel device. To characterize the performance of the dry-film resist, a 50 μm-thick film was used to generate 230 μm-wide PDMS channels. (B) Scanning electron microscopy (SEM) image of the dry-film photoresist master structure (top view). (C) A top- and side-view SEM image of the PDMS device cast from the master structure shown in (B). The images revealed that the actual channel width was 235±2 μm (width ± standard deviation; also see Table S1). SEM imaging was used to measure the reproducibility of the devices made by molding PDMS around a dry-film photoresist. To ascertain the reproducibility of the fabrication process, three devices were cast from three different masters. In addition, three PDMS devices were cast from the same master. Images of these six devices were used to estimate the width (W) and height (H) of the main channel. As summarized in Table S1, the devices were very reproducible, even with reusing the same dry-film master up to three times. Scale bars are 50 μm.

Table S1. Reproducibility of the Width and Height of the Y-Channel Device

	Central Channel Width (μm)*	Central Channel Height (μm)*
Using different masters (N=3)	235 ± 2	49 ± 1.2
Using the same master (N=3)	234 ± 3	49 ± 1

*Numbers indicate mean ± standard deviation of three different devices.

Linear Gradient Device

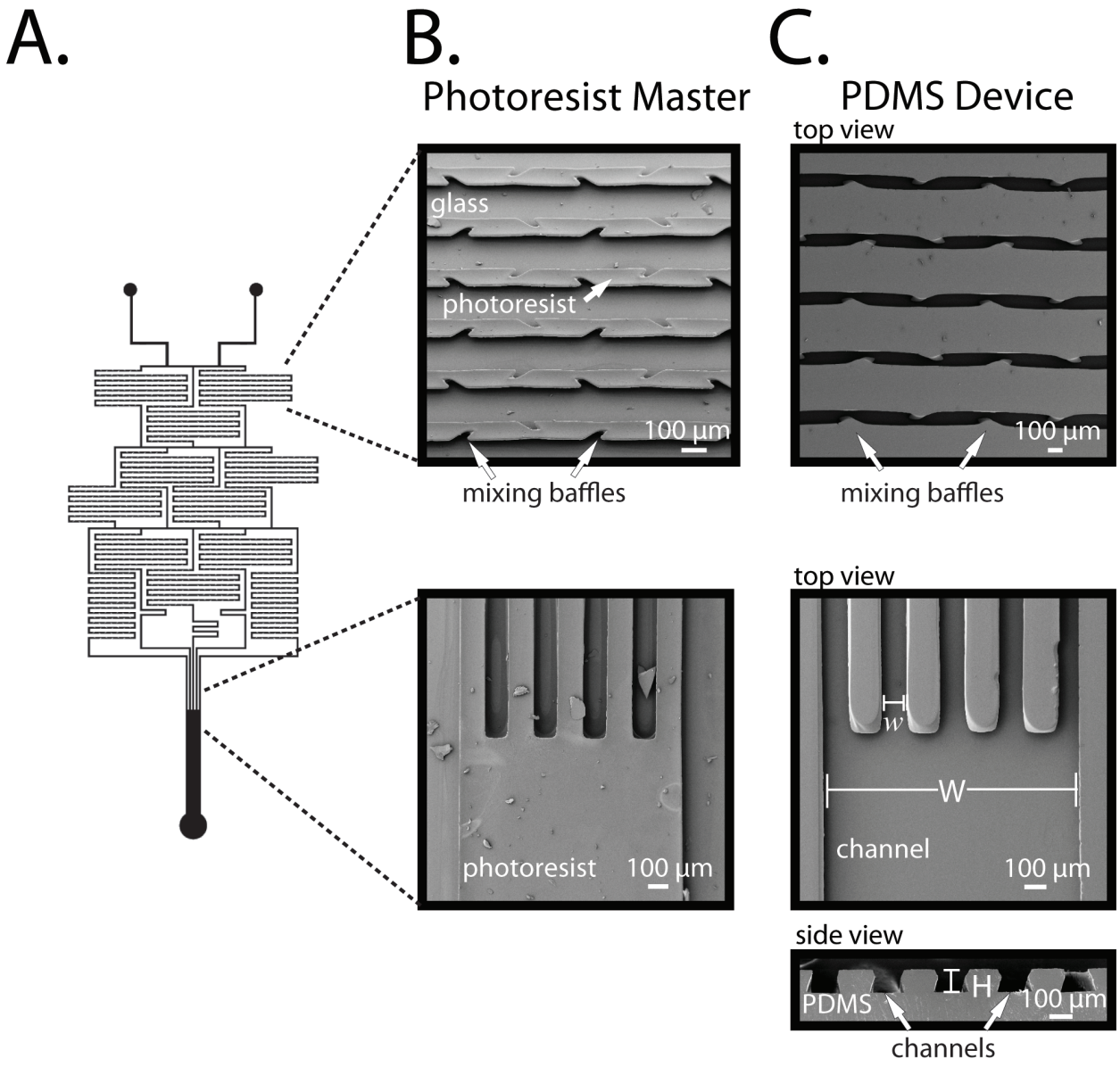

Figure S2. Characterization of the gradient mixer. (A) Schematic design for the linear gradient mixer. (B) Scanning electron microscopy (SEM) images of the dry-film photoresist master structures (top view). (C) A top- and side-view SEM image of the PDMS device cast from the master structures shown in (B). SEM images of the mixing baffles are shown in the top two panels, whereas the lower channels that are used for fluorescence imaging are shown in the bottom two panels. As for the Y-channel device, SEM imaging was used to measure the reproducibility of the gradient mixer devices. Three PDMS molds were cast from three different masters. In addition, three PDMS molds were cast from the same master structure. Images of these six devices were used to estimate the width of the main channel (W), the width of the individual imaging channels (*w*) and the height of the channel (H). These results are summarized

in Table S2. As before, device fabrication was very reproducible, even when re-using a master structure for multiple PDMS devices. Glass shards visible in (B) were introduced when the master structures were cut down in size to fit within our SEM imaging chamber. Scale bars are 100 μm.

Table S2. Reproducibility of the Width and Height of the Gradient Mixer Device

	Individual Channel Widths, w (μm)*	Central Channel Width, W (μm)*	Central Channel Height, H (μm)*
Using different masters (N=3)	107 ± 3	1110 ± 0.6	90 ± 1.4
Using the same master (N=3)	108 ± 3	1120 ± 10	90 ± 0.4

*Numbers indicate mean ± standard deviation of three different devices.

2. Measuring DNA Extension

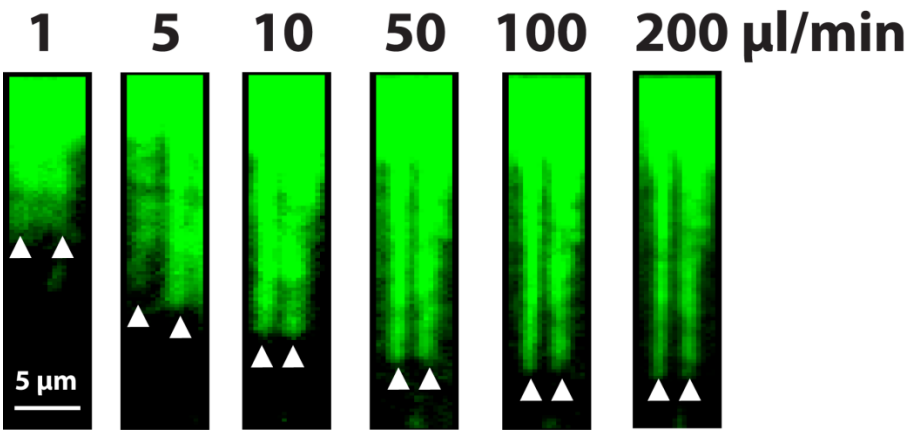

Figure S3. Representative fluorescent images of two λ-DNA molecules as a function of the buffer flow rate. The lengths of the DNA molecules were measured in ImageJ. The white triangles indicate the ends of the DNA. Increased signal at the top of each panel is due to accumulation of fluorescent dye in the scratches on the glass substrate used to create the DNA curtains.

3. Characterizing the Gradient Mixer Device Performance

Fluorescein as Analyte

Table S3 summarized the reproducibility of fluorescein mixing in the microfluidic gradient generator as a function of the flow rate. Fluorescein was dissolved in imaging buffer (10 mM Tris-HCl [pH 8], 100 mM NaCl, 0.2 mg ml^{-1} BSA) to a final concentration of 50 nM. One of the two microfluidic ports was connected to a syringe delivering 50 nM (100%) fluorescein, whereas the second syringe delivered imaging buffer without fluorescein (0%). Epifluorescent measurements were taken across each channel using a 10x objective (Nikon). The signal from a channel without any analyte was subtracted from all the other channels. Then, all channels were normalized against the 100% channel (50 nM fluorescein). The 0% channel was further verified to not contain any fluorescein by comparing its fluorescence signal to a device that has never been exposed to fluorescein. Likewise, the fluorescence in the 100% channel was verified by comparison to a device filled completely with 50 nM fluorescein. Errors bars represent the standard deviation of at least three measurements taken using three devices. These values are represented in graphical form in Figure 6c of the main text.

Table S3. Fluorescein forms gradients in the microfluidic mixer.

	Channel 1 (% solute)	Channel 2	Channel 3	Channel 4	Channel 5
10 μl min^{-1}	0	25 ± 2.3	50 ± 2.4	73 ± 1.3	100
25 μl min^{-1}	0	22 ± 0.30	50 ± 0.43	77 ± 0.13	100
50 μl min^{-1}	0	20 ± 0.04	50 ± 0.59	79 ± 0.47	100
75 μl min^{-1}	0	21 ± 0.26	50 ± 0.70	77 ± 0.21	100
100 μl min^{-1}	0	23 ± 0.61	50 ± 1.7	76 ± 1.1	100

Error bars report the standard deviation of three measurements taken with three different devices.

Protein as Analyte

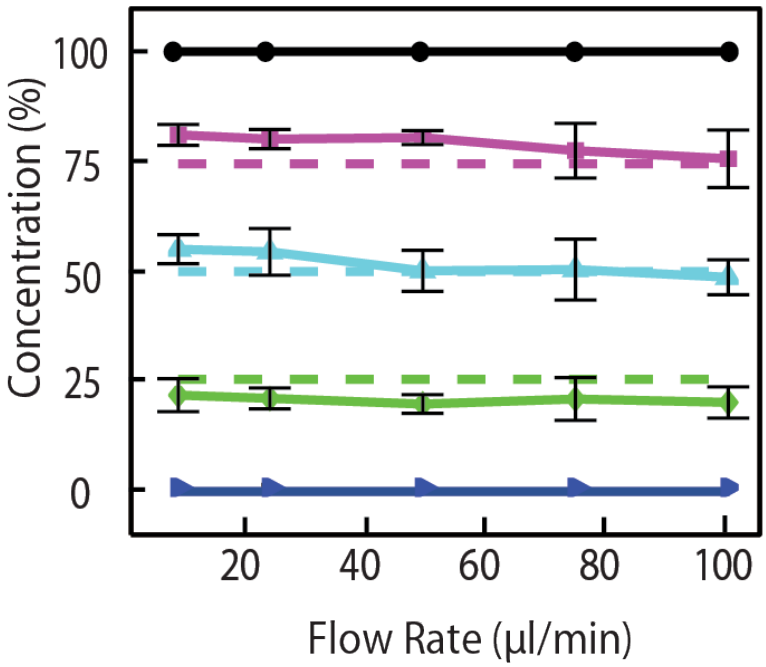

Figure S4. Human Replication Protein A (RPA)-GFP forms gradients in the microfluidic mixer. The mixing efficiency for each channel is plotted as a function of the flow rate. These values are also summarized in Table S4. Devices were passivated with lipid bilayers as described in the main text to reduce adsorption of protein in the channels. Measurements were taken across at least three devices and the error bars report the standard deviation of three measurements across three different devices. The dashed lines represent the expected concentrations produced by an ideal linear gradient-generating device.

RPA-GFP has an approximately ~10 nm Stokes radius,[2,3] and is a much larger analyte than fluorescein (Stokes radius of ~0.5 nm).[4] As with Figure 6c (fluorescein mixing characterization), the signal without any fluorescent analyte was used for background subtraction from all channels. The intensities in the first four channels were normalized against the 100% analyte channel (corresponding to 50 nM hRPA-GFP).

Table S4. hRPA-GFP mixing efficiency as a function of the flow rate.

	Channel 1 (% analyte)	Channel 2	Channel 3	Channel 4	Channel 5
10 μl min^{-1}	0	21 ± 3.8	55 ± 3.4	82 ± 2.4	100

25 μl min^{-1}	0	21 ± 2.4	55 ± 5.4	81 ± 2.2	100
50 μl min^{-1}	0	19 ± 2.1	50 ± 4.7	81 ± 1.7	100
75 μl min^{-1}	0	20 ± 4.9	51 ± 7.0	78 ± 6.3	100
100 μl min^{-1}	0	20 ± 3.6	49 ± 4.0	78 ± 6.6	100

Error bars report the standard deviation of three measurements taken with three different devices.

4. Supplemental References

(1) Modesti, M. In *Single Molecule Analysis*; Peterman, E. J. G.; Wuite, G. J. L., Eds.; Humana Press: Totowa, NJ, 2011; Vol. 783, pp. 101–120.
(2) Kim, C.; Paulus, B. F.; Wold, M. S. *Biochemistry* **1994**, *33*, 14197–14206.
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(4) Banks, D. S.; Fradin, C. *Biophys. J.* **2005**, *89*, 2960–2971.