A Barcode-Free Combinatorial Screening Platform for Matrix Metalloproteinase Screening

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Section 1: Microfluidic device fabrication

The microfluidic devices for our experiments were fabricated using standard multilayer soft-lithography techniques. The fluidic layer mold for the devices was fabricated through dual layer photolithography. Initially a layer of SPR-220 7.0 (MicroChem Corp.) photoresist is patterned on a 4 inch silicon wafer. This positive photoresist was used to generate the channel regions on the device to be compressed by the control layer. Following this step, a layer of SU8-3025 (MicroChem Corp.) was patterned on the same wafer to generate the rest of the fluidic layer network on the device. An independent control layer mold was prepared on a separate silicon wafer by patterning a single layer of SU8-3025 on the wafer. These molds were then used to fabricate devices using multi-layer soft lithography. Initially a ~1mm thick layer of polydimethylsiloxane (Sylgard Elastomer 184, Ellsworth Adhesives, 6:1 base to curing agent ratio) was spin-coated on the control layer mold. This layer was partially cured in an oven at ~80°C for 5-6 minutes. The PDMS membrane on the mold was then peeled off and trimmed to prepare for alignment with the fluidic layer. A thin layer of PDMS was spin coated on the fluidic layer mold and partially cured in the oven at ~80°C for 3-4 minutes. The PDMS membrane from the control layer mold was then aligned with the fluidic layer using a stereoscope. These two layers were allowed to bond for 10 minutes at ~80°C. Following this, ~50g of PDMS (10:1 base to curing agent ratio) was poured on these two layer to form the thick body of the device. This thick PDMS layer was allowed to cure for at least 25 minutes at ~80°C. The devices were then peeled off the mold and holes were punched at the input and output ports. The devices were bonded to Thickness #1 cover glass (Ted Pella, Inc.) using oxygen plasma treatment. Before use, the devices were treated with Aquapel (PPG Industries) to render the fluidic channels on the device hydrophobic.

Section 2: Peptide substrate sequences

SB2: QXL520TM -Pro-Leu-Ala-Leu-Trp-Ala-Arg-Lys(5-FAM)-NH2
SB3: QXL520 TM -Pro-Leu-Gly-Cys(Me)-His-Ala-D-Arg-Lys(5-FAM)-NH_2
SB4: 5-FAM-Pro-Leu-Ala-Nva-Dap(QXL520 TM)-Ala-Arg-NH_2
SB5: 5-FAM-Pro-Leu-Gly-Leu-Dap(QXL520 TM)-Ala-Arg-NH_2
SB8: QXL520 TM -Arg-Pro-Lys-Pro-Leu-Ala-Nva-Trp-Lys(5-FAM)-NH_2
SB9: QXL520 TM -Arg-Pro-Leu-Ala-Leu-Trp-Arg-Lys(5-FAM)-NH2
SB11: 5-FAM-Pro-Cha-Gly-Nva-His-Ala-Dap(QXL™520)-NH_2
SB12: 5-FAM-Arg-Pro-Lys-Pro-Tyr-Ala-Nva-Trp-Met-Lys(QXL520 TM)-NH2
SB15: QXL520 TM -γ-Abu-Pro-Gln-Gly-Leu-Dab(5-FAM)-Ala-Lys-NH_2

Section 3: Determining maximum reagent dilution on device

To generate maximum reagent dilution on the device, we tested the smallest reagent injection possible on our device. To conduct this experiment we generated various dilutions of Alexa Fluor 546 dye on our device. The Alexa Fluor 546 input was maintained under a low pressure of 1psi while the buffer input was maintained at a high pressure of 10psi. We then generated a droplet sequence which included droplets containing Alexa Fluor 546 injections generated using valve opening times of 0.0125, 0.05, 0.0875, 0.125, 0.1625, 0.2, 0.2375 and 0.275 seconds for the valve corresponding to the Alexa Fluor 546 input. We also included a buffer-only droplet at the beginning of the droplet sequence as well as an Alexa Fluor 546 droplet without any dilution at the end of the sequence. Fluorescence data collected from four repeats of this droplet sequence can be seen in Figure S1. We observed that a valve opening time of 0.0875 seconds was necessary to observe reliable injection of reagent into the droplets. The maximum dilution of the dye obtained for this valve opening time was 0.0066X, calculated as the ratio of the average fluorescence intensity of the droplet corresponding to 0.0875 second valve opening time to the average fluorescence intensity of the undiluted Alexa Fluor 546 droplet at the end of the droplet sequence.

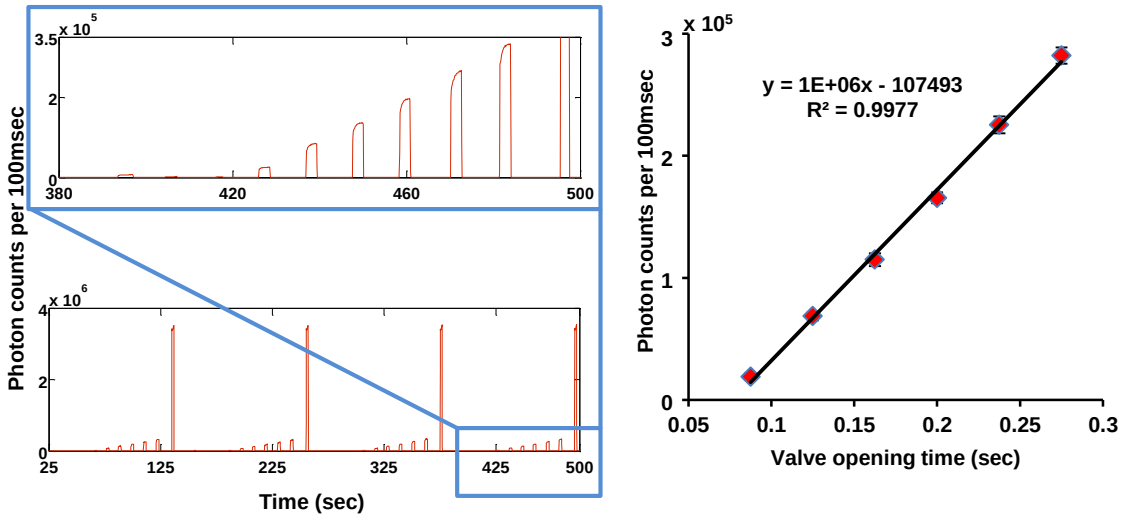

Figure S1. Largest reagent dilution generated on the device: Left panel: The data trace indicates four repeats of a sequence of droplets generated to test the largest reagent (Alexa Fluor 546) dilution that can be produced on our device. Each sequence ends with an undiluted reagent droplet to indicate the scale of dilution. **Right panel:** Average droplet fluorescence intensities are plotted against the valve opening times here. The linear relationship indicates predictable droplet composition control through simple valve opening time control. Error bars indicate standard deviation over four repeats of the droplet sequence. The smallest reagent injection volume on the device was calculated to be 60pL from this data.

Section 4: Fluorescence standard curve

Various concentrations (2, 1, 0.5, 0.25, 0.125, 0.0625, 0.03125 and 0.015625 µM) of the fluorescence reference standard included in the MMP substrate sampler kit were used to generate a standard curve relating fluorescence intensity with substrate concentration. To generate the standard curve for the optical setup used with the microfluidic device, a train of droplets was generated using different concentrations of the reference standard for fluorescence measurement to closely mimic the experimental conditions on the device. Excellent linear relation was observed between reference standard concentration and the measured fluorescence intensities (Figure S2).

Similarly fluorescence intensities of the same set of concentrations of the reference standard were measured on the CFX96 Real-Time PCR detection system used for fluorescence monitoring from off-chip experiments. The plot of the measured fluorescence intensities against the corresponding reference standard concentrations shows linear relation between the two variables (Figure S2).

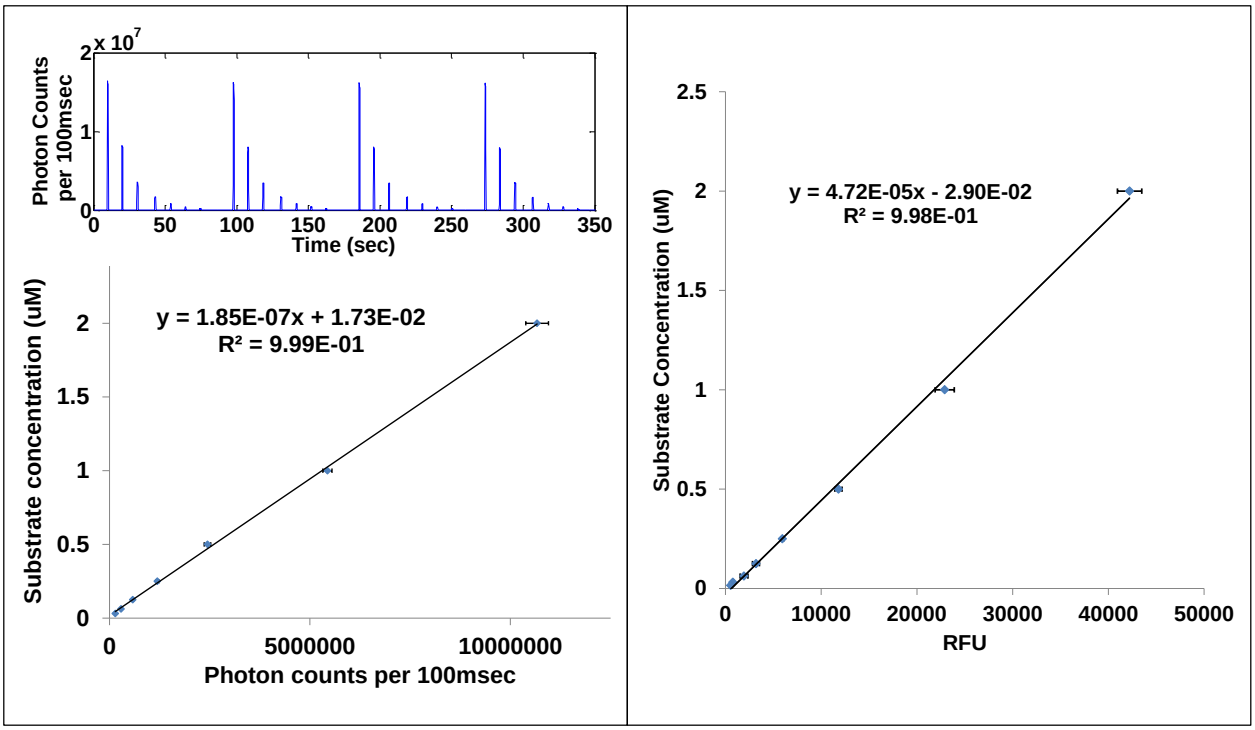

Figure S2: Standard curves. Left panel: The data trace on top shows four repeats of a droplet sequence generated on device with different concentrations of fluorescent substrate reference standard. The average fluorescence intensity data extracted from this droplet sequence is plotted against the known reference standard concentrations in the droplets in the bottom plot to generate a standard curve for on-chip experiments. **Right panel:** A plot of measured fluorescence intensity (on the CFX96 Real-Time PCR detection system) against corresponding concentrations of the reference standard.

Section 5: Additional data for on-chip vs off-chip MMP activity comparison

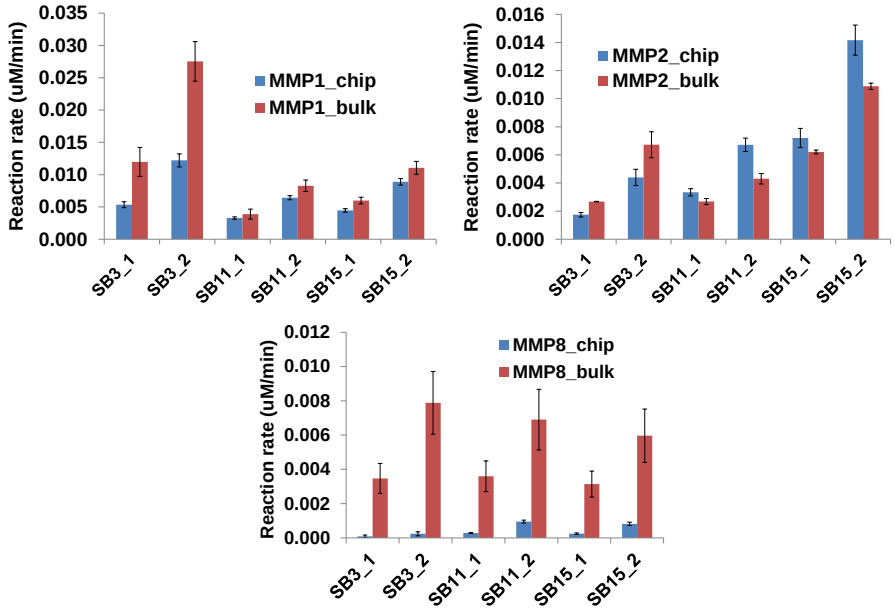

Figure S3. **MMP activity comparison between on and off-chip experiments:** (**MMP1_chip:** MMP1 at 1X concentration on-chip, **MMP1_bulk:** MMP1 at 1X concentration off-chip, **SB3_1:** Substrate SB3 at 1X concentration)

Section 6: Long droplet sequence generation on device

As a proof of principle, we generated a large sequence of droplets containing 650 unique MMP-substrate combinations on a single device. Average fluorescence intensities obtained from three repeats of droplets corresponding to each unique combination are shown in Figure 6 in the main text. Plots of measured fluorescence intensities from different MMP-substrate combinations against each other for different repeats show good linear correlation indicating repeatable operation of the device over long periods of time (Figure S4).

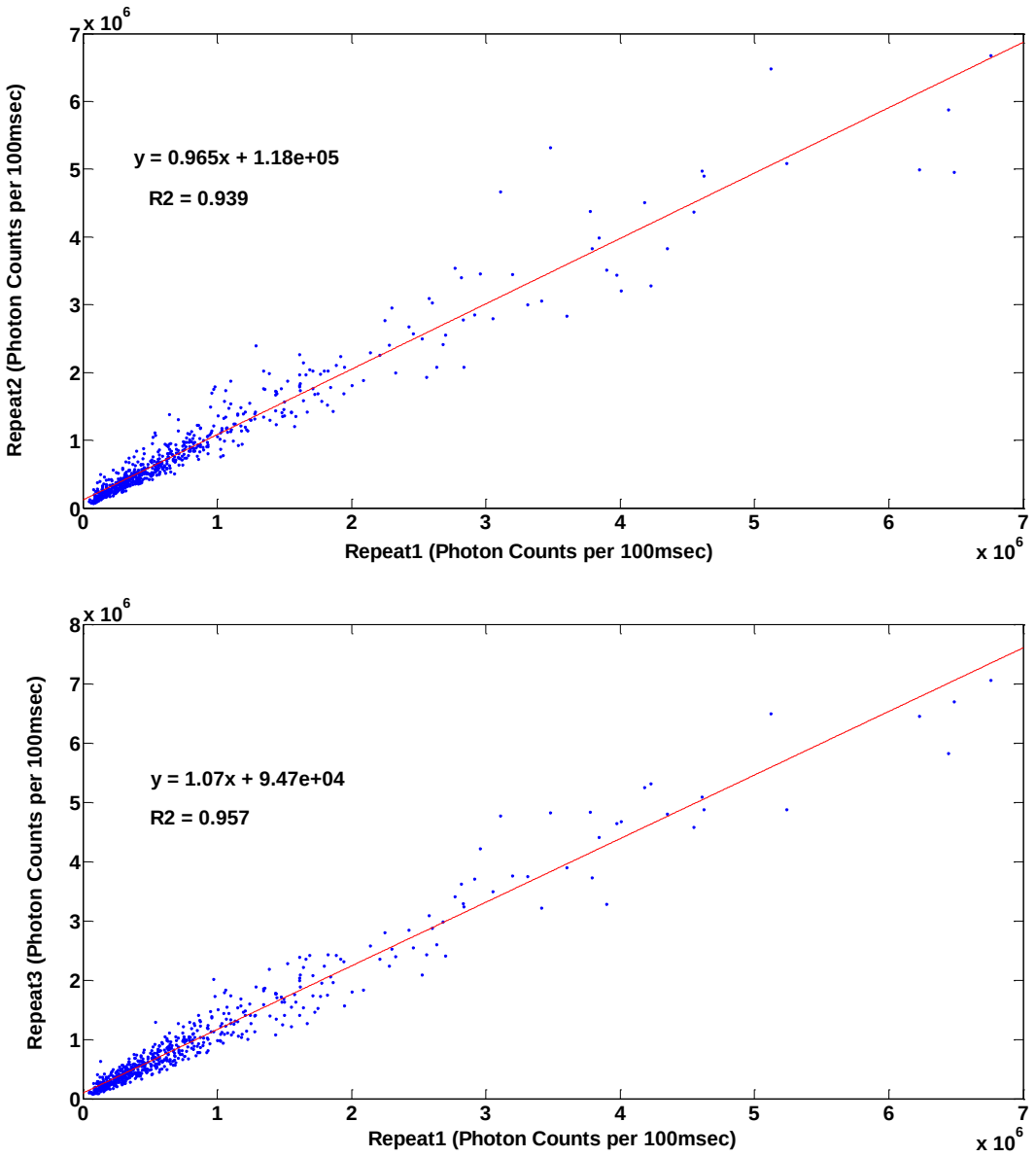

Figure S4: Average fluorescence intensities measured from different repeats for each unique MMP-substrate combination plotted against each other show good linear correlation indicating repeatable operation of the device over long periods of time.