Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Brightfield and fluorescence in-channel staining of thin blood smears generated in a pumpless microfluidic

Kokou S. Dogbevi [a] Paul Gordon,[a*] Kimberly L. Branan,[a] Bryan Khai D. Ngo,[a] Kevin B. Kiefer,[d] Susanne U. Martens-Talcott,[d] Melissa A. Grunlan,[a,b,c,e] Gerard L. Coté [a,e,**]*

[a] Department of Biomedical Engineering, Texas A&M University, College Station, TX 77843, USA. E-mail: gcote@tamu.edu

[b] Department of Materials Science & Engineering, Texas A&M University, College Station, TX 77843, USA

[c] Department of Chemistry, Texas A&M University, College Station, TX 77843, USA

[d] Department of Nutrition and Food Science, Texas A&M University, College Station, TX 77843, USA

[e] Center for Remote Health Technologies & Systems, Texas A&M University, College Station, TX 77843, USA

Part A: Fabrication of microfluidic channels

Materials. Allyl methyl PEO (Polyglykol AM 450, M_n = 292–644 g mol^{-1} per manufacturer's specifications; M_n = 424 g mol^{-1} per proton (^1H) nuclear magnetic resonance (NMR) end group analysis) was provided by Clariant. Octamethylcyclotetrasiloxane and tetramethyldisiloxane were purchased from Gelest. $ODMS_{30}$ (M_n = 2354 g mol^{-1} per ^1H NMR end group analysis) was prepared as reported.[1] Triflic acid, rhodium (I) tris(triphenylphosphine) chloride (Wilkinson's catalyst), hexamethyldisilazane, tridecafluoro-1, 1, 2, 2-tetrahydrooctyl-1-trichlorosilane, and solvents were obtained from Sigma-Aldrich. All solvents were dried over 3 Å molecular sieves prior to use. Sylgard 184 was purchased from Ellsworth Adhesives, and SU8-5 from Microchem. Glass microscope slides (75 × 25 × 1 mm) were purchased from Fisher Scientific.

Synthesis of PEO-silane amphiphile ($HSi\text{-}PDMS_{30}\text{-}PEO_8\text{-}OCH_3$). The PEO-silane amphiphile $HSi\text{-}PDMS_{30}\text{-}PEO_8\text{-}OCH_3$ was synthesized as previously reported using a Wilkinson's-catalyzed regioselective hydrosilylation reaction of $ODMS_{30}$ and allyl methyl PEO_8 (1:1 molar ratio).[1] ^1H NMR of the purified product, a clear and colorless liquid, was in agreement with that previously reported.

Mask design and fabrication. A standard SU8-5 process was used to fabricate the master mold[2] and having a design mask design depicted in **Figure S1** as per our prior report.[3] The design included four identical microfluidic channels that were 16 mm long (from the center of the inlet to the center of the outlet), 300 μm wide and 4.7 μm in height. A series of pillars (50 μm diameter, 25 μm gap between pillars, ~4-5 horizontal rows) were included at four different locations along the length of the channel: at the inlet, at the outlet, as well as at two locations approximately 4 mm and 8 mm from the inlet. The series of pillars at the inlet and outlet spanned a length of ~1.2 mm

and was comprised of ~17 vertical columns of pillars. The interior series of pillars occupied a length of ~184 μm and also consisted of ~3 vertical columns of pillars. The Dektak profilometry was used to measure the channel height. After the master mold microfabrication process, the master mold was coated with tridecafluoro-1, 1, 2, 2-tetrahydrooctyl-1-trichlorosilane for easy removal of the mold.

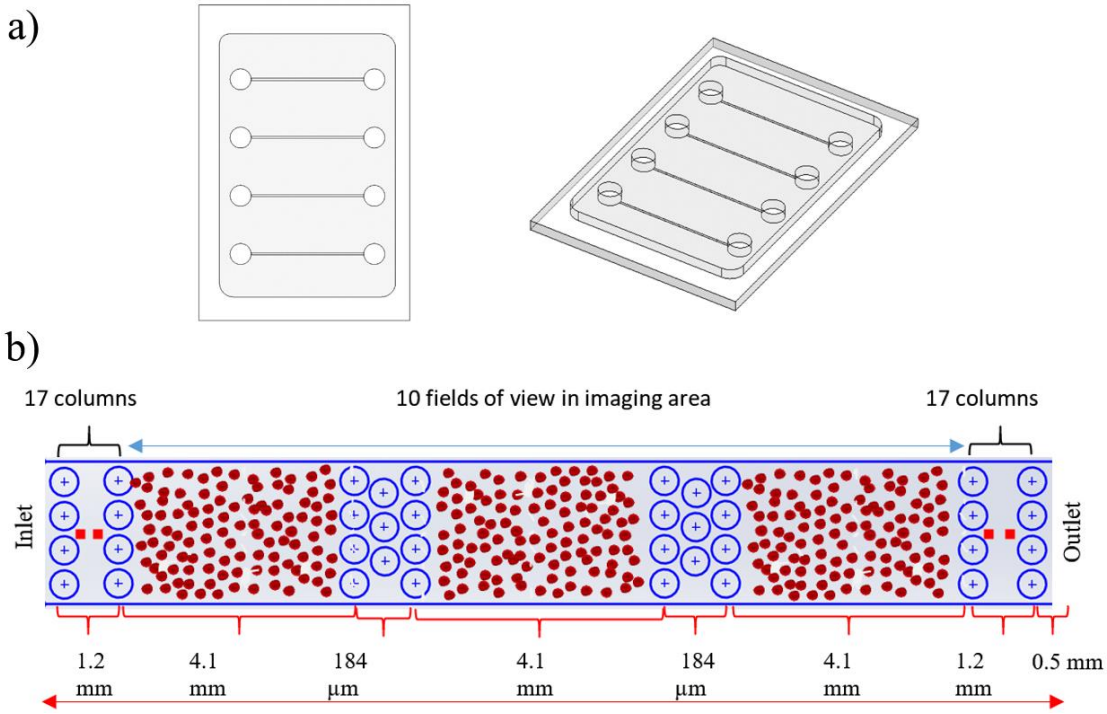

Figure S1. A) Microfluidic chip overall device design. B) Microfluidic chip mask design. *Blue circles*: Pillars (diameter ~45 μm; gap distance ~25 μm); *Red Circles*: Red blood cells (RBCs). *Inlet & outlet:* Pillar sections are comprised of 30 columns and 4-5 rows of pillar. *Interior*: Pillar sections are comprised of 3 columns and 4-5 rows of pillars. Channel height was maintained at 4.7 μm and channel width at 300 μm.

Microfluidic chip fabrication. Sylgard 184 "base" was added with the Sylgard 184 "curing agent" at a 10:1 weight ratio. To this was added 7 wt% HSi-$PDMS_{30}$-PEO_8-OCH_3 (based on the total weight of the Sylgard base and curing agent). The samples were mixed in a FlackTeck speed mixer for 15 sec at 1000 rpm, then manually ramped to 3000 rpm over 15 sec, and mixed at 3000 rpm

for 4 min. After that, the sample was removed from the mixer and manually mixed while placed in a 70 °C water bath before pouring into a 60 °C pre-heated master mold. The sample was immediately desiccated for 13 min (36 mm Hg) to remove the air bubbles and cured at 130 °C for 1.5 hr. The cured molds were removed from the master molds and stored for 2-5 days at room temperature (RT). During this period, circular wells (5 mm diameter) were created at the inlet and outlet with a biopsy punch. This reduced the channel length to ~14 mm and the series of pillars to ~20 mm. The resulting molds were bonded to glass using an oxygen oven plasma cleaner (Harrick Plasma PDC-001) with the following protocol: sequential vacuum (10 min), oxygen (2 min), plasma (2 min), and 110 °C oven (2 hr). The resulting microfluidic chips contained four equivalent channels (~300 μm wide, ~4.7 μm height, ~13 mm long), D-shaped channels with the curved portion (facing the bottom) made of the amphiphilic silicone and the flat portion (facing the top) was the glass. The bonded molds (chip) were stored in air at RT for 24 hr prior to use to permit the plasma effect to dissipate from the chip.

Part B: Reconstitution of malaria cultures to replicate parasitized human blood

P. falciparum malarial parasites were cultured asynchronously *in vitro* to a parasitemia of approximately 5% in human RBC's. To ensure that the cultured cells used during testing would behave consistently with whole blood that would be collected during practical use, the parasitized RBC's were reconstituted with whole blood from the same donor. Both in-channel and glass mounted smears were created during each stage of culture reconstitution to assess smear performance, with results shown in **Figure S2**. Samples of the cultured cells suspended in growth media were centrifuged for 2 min at 2,000 rcf to form a loose pellet of parasitized RBC's in the base of the tube. The supernatant media was removed, and the process repeated twice more to form a non-packed, un-lysed collection of parasitized RBC's. This pellet was resuspended 1:1 by

volume in fresh human blood plasma, and the resulting resuspended culture was mixed 1:1 with whole blood to create a physiologically accurate sample of human blood doped with *P. falciparum*. The human blood used for parasite culture and sample reconstitution was donated under exemption approval from the Texas A&M Institutional Review Board. Control smears from each stage of sample reconstitution were created and examined to ensure that cellular morphology and hematocrit were preserved.

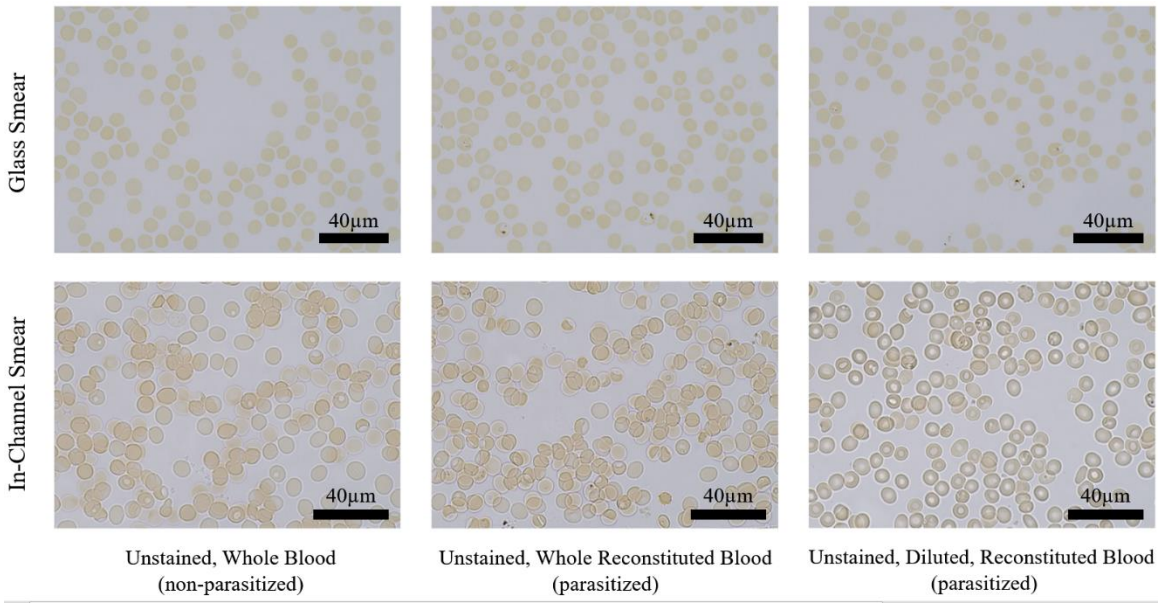

Figure S2: Assessment of reconstitution quality.

Part C: Inter-channel repeatability and effect of varying fluorescent Acridine Orange stain concentrations during in-channel staining

Channels stained using 6 µg/mL, 12 µg/mL, and 96 µg/mL Acridine Orange were collected to assess staining quality for several concentrations of stain. Results show the relationship between increased stain concentration and fluorescent intensity, with the highest concentration of stain

showing comparable parasitic intensity to that of the gold-standard images with much greater contrast between parasites and background.

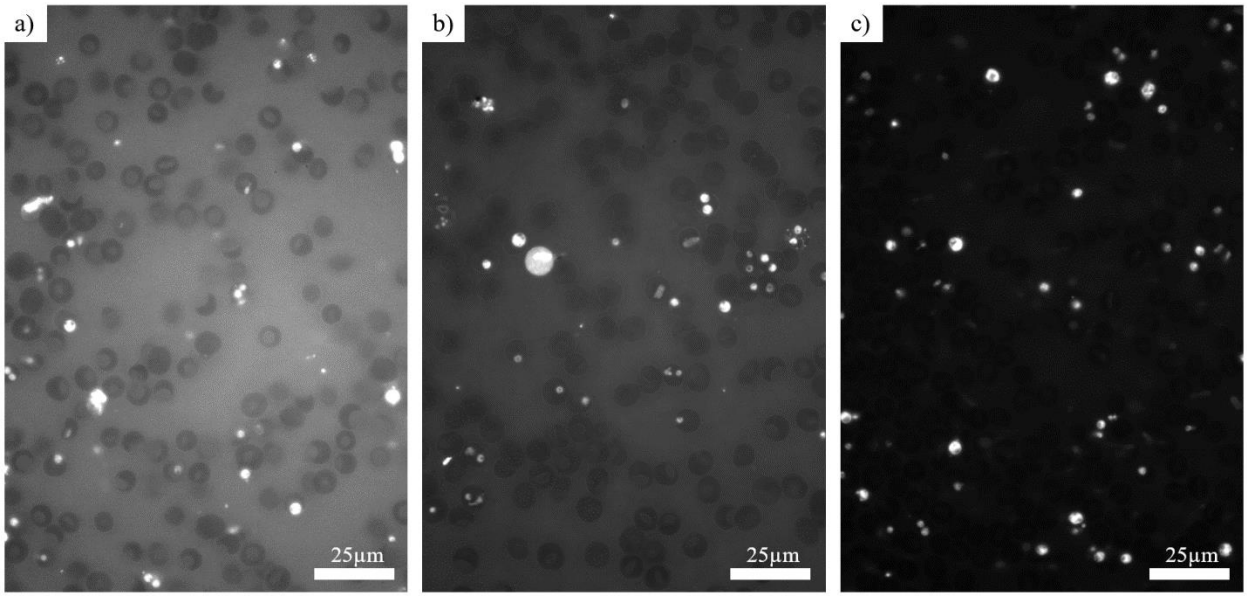

Figure S3: Monochromatic images of in-channel samples fluorescently stained using a) 6 µg/mL, b) 12 µg/mL, and c) 96 µg/mL Acridine Orange staining solutions. Greater contrast between parasites and background can be seen with higher stain concentration. Images shown with linear contrast enhancement for visibility.

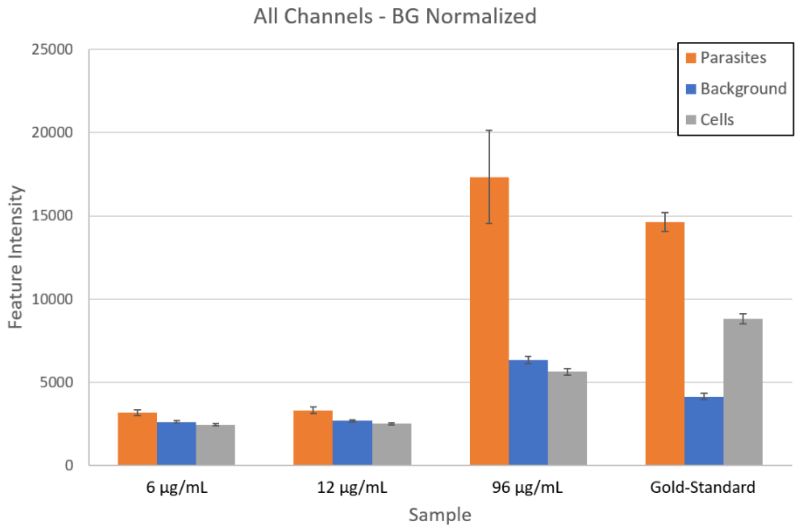

Figure S4: Values of fluorescent feature intensity as functions of Acridine Orange stain concentration for in-channel samples and glass smear ("gold standard").

References

1. Rufin, M. A.; Ngo, B. K. D.; Barry, M. E.; Page, V. M.; Hawkins, M. L.; Stafslien, S. J.; Grunlan, M. A., Antifouling Silicones Based on Surface-Modifying Additive Amphiphiles. *Green Mater* **2017,** *5* (1), 1-10. DOI: 10.1680/jgrma.16.00013.

2. Thangawng, A. L.; Swartz, M. A.; Glucksberg, M. R.; Ruoff, R. S., Bond-Detach Lithography: A Method for Micro/Nanolithography by Precision Pdms Patterning. *Small* **2007,** *3* (1), 132-138. DOI: 10.1002/smll.200500418.

3. K. S. Dogbevi, B. K. D. Ngo, K. L. Branan, A. M. Gibbens, M. A. Grunlan, G. L. Coté "A thin whole blood smear prepared via a pumpless microfluidic," *in review*.