Electronic Supplementary Material (ESI) for Lab on a Chip.

"Spatially Isolated Reactions in a Complex Array: Using Magnetic Beads to Purify and Quantify Nucleic Acids with Digital and Quantitative Real-time PCR in Thousands of Parallel Microwells"

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Microchips for 25 and 5 pL reactions were fabricated from silicon wafers and glass substrates using photolithography and deep reactive ion etching (DRIE). Briefly, 150 mm diameter, ≈ 600 µm thick test grade silicon wafers (University Wafer, part #857) were coated with ≈ 5 nm of titanium and then ≈100 nm of gold using either electron beam evaporation (Thermionics VE-100) or metal sputtering (Kurt Lesker PVD 75). Positive photoresist (Shipley Microposit S1813, Microchem Corp., Westborough, MA) was exposed through a photomask and developed to reveal the reaction region and channel wall pattern. The revealed areas were then etched with aqua regia to remove the gold and expose the titanium. B270 glass substrates (127 mm x 127 mm x 0.9 mm) were coated with ≈ 5 nm of titanium and ≈ 20 nm of gold. The glass was patterned and etched to remove the gold in the reaction regions (50 µm squares for 25 pL chips and 25 µm diameter circles for 5 pL chips) using the same process as the silicon wafer. The gold was thin enough such that it was partially transparent. Vias were drilled in the glass using abrasive powder blasting (Comco MicroBlaster).

The silicon was again coated with positive photoresist and patterned for the bead wells. DRIE (Alcatel AMS 100) was used to etch an array of ≈ 4-4.2 µm diameter, 4.5-5 µm deep, cylindrical pits through the titanium layer and into the silicon in the center of the reaction regions using a low roughness Bosch process. Oxygen plasma (≈500 W) was used to strip the remaining photoresist, and the wafer was coated with ≈10 µm of KMPR 1010 negative photoresist. A photomask was used to pattern the KMPR to form the sidewalls that sealed the microfluidic chip as well as flow guides that partition areas of the array into a series of parallel channels. After development of the resist, the wafer was descummed using oxygen plasma to remove trace photoresist residue from the reaction regions.

The wafer and the glass substrate were coated with positive resist to protect them from debris, and diced into individual chips (Basic Dicer II). After washing with 5% Contrad solution, the resist was then stripped using acetone and isopropyl alcohol. A small amount of a two-part epoxy (Loctite E-120HP, Henkel America) was used to bond the glass to the silicon layer after alignment of the etched reaction region patterns using a Finetech Fineplacer Lambda flexible bonder. Bonded chips were heated to 65 °C overnight to fully cure the epoxy.

A saturated solution of 1-octadecanethiol was prepared in ethanol and allowed to sit for at least 24 hours before it was filtered through a 0.22 µm PTFE syringe filter. Chips were filled with and then submerged in this solution and left for 24 hours to allow formation of a self-assembled monolayer of 1-octadecanethiol on the gold surfaces of the chip. The following day, the chips were rinsed with pure ethanol, heptane, and then pure ethanol again before use.

Microchips for 100 fL reactions were fabricated in a different manner. Briefly, 150 mm diameter, ≈ 600 µm thick test grade silicon wafers were coated with ≈ 100 nm of chrome and ≈ 500 nm of positive photoresist (Nanofilm, Valley View, Ohio). Microwell patterns were written directly onto the photoresist using a DWL 66fs laser writer (Heidelberg Instruments, Heidelberg Germany). The chrome was etched to expose the silicon surface before DRIE etching using a low roughness Bosch process to create the microwell geometries. Oxygen plasma was then used to remove the photoresist before the chrome layer was stripped with chromium etchant. SU8 2050 (Microchem Corp.) was then patterned to create a chamber border and fluid flow control "lanes" from 20 to 45 µm thick. After development of the SU8, the wafer was coated with a protective layer of positive photoresist before being diced. After dicing, the photoresist was stripped and the chips were cleaned with 5% Contrad solution.

A 127 mm x 127 mm x 0.9 mm B270 glass substrate was coated with a protective layer of photoresist. Vias were drilled into the glass using abrasive powder blasting. The substrate was then diced to form coverslips for the silicon chips. E-120HP epoxy was used to bond the glass covers to the silicon substrates and cured at 65 °C for at least 12 hours. The chips were rinsed with ethanol and dried. A 1% solution of octyltrichlorosilane in dry heptane was pipetted into the chip until the array chamber was filled. After approximately 30 s, the chip was emptied until dry using vacuum. The chip was placed in a drying oven at 65 °C for at least 1 h before storage.

SIRCA Reagents and Samples

Early work was performed with extracted and purified genomic DNA purchased from ATCC (Manassas, Virginia): methicillin-susceptible *Staphylococcus aureus* (MSSA, ATCC 25923), methicillin-resistant *S. aureus* (MRSA, ATCC 700699), and *Streptococcus mutans* (ATCC 25175). The gDNA was digested with restriction enzymes to fragment the DNA into smaller lengths. A typical digestion reaction contained 50% gDNA solution (20 µg/mL), 25% water, 1% of EcoRV-HF, 4% Bpu10I, 10% SfaNI enzymes (New England Biolabs, Ipswich, MA), and 10% NEB buffer 3. The mixture was gently mixed and incubated at 37 °C for 15 min before inactivating the enzymes by heating to 95 °C for 5 min. Primer sequences for *nuc*, *mecA*, and *S. mutans* were based upon those reported by Oblath et. al.[1] Primers for the RNA and DNA sequences used to quantify malaria parasites were based upon those reported by Kamau et al.[2] Modifications to these primers includes the addition of the 5′ flap sequence reported by Afonina et. al.[3]

Table S1. shows the number of wells, the well volume, the number of beads incubated with the sample, the number of beads that loaded into the chip as single beads in one well, the loading efficiency, and the bead utilization (i.e. the percentage of beads incubated with the sample that were analyzed) averaged over the chips used for each experiment. Note that the number of wells imaged for each of the 100 fL chips (≈18,725) was less than the total number of wells on the chip (>30k), due to a limited field of view. We expect that tightening tolerances of the 100 fL well geometry will significantly improve bead loading efficiency and a higher resolution camera will permit a larger field of view.

Table S1.

	# of wells	well volume	# of beads	# of beads loaded	loading efficiency	bead utilization
Fig. 2.	1,500	25 pL	18,000	1,103	73.5%	6.1%
Fig. 3.	6,000	4.2 pL	175,000	4,467	74.4%	2.6%
Fig. 5.	6,000	4.2 pL	175,000	4,434	73.9%	2.5%
Fig. 7.	18,725	100 fL	200,000	11,362	60.7%	5.7%
Fig. 8.	18,725	100 fL	200,000	11,573	61.8%	5.8%

REFERENCES

(1) Oblath, E.A., Henley, W.H., Alarie, J.P., Ramsey, J.M.; *Lab on a Chip* **2013**, 1325-1332.
(2) Kamau, E.; Tolbert, L. S.; Kortepeter, L.; Pratt, M.; Nyakoe, N.; Muringo, L.; Ogutu, B.; Waitumbi, J. N.; Ockenhouse, C. F. *Journal of Clinical Microbiology* **2011**, *49*, 2946-2953.
(3) Afonina, I.; Ankoudinova, I.; Mills, A.; Lokhov, S.; Huynh, P.; Mahoney, W. *BioTechniques* **2007**, *43*, 770-774.

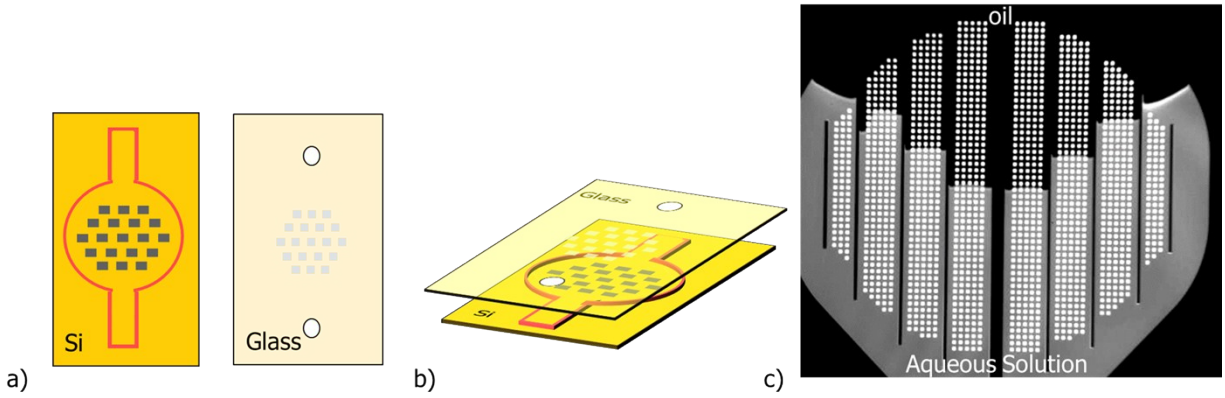

Figure S1. 25 and 4.2 pL chips. a) Devices were fabricated using gold coated silicon and glass substrates that were etched to reveal hydrophilic regions (grey squares). A bead well was etched into each hydrophilic region on the silicon substrate. Photopatterned epoxy (red) was used to create sidewalls and flow control channels. b) The glass was aligned and bonded to the silicon substrate. c) A chip filled with aqueous solution containing a fluorescent dye during oil sealing. Aqueous phase is trapped at the hydrophilic "windows", where the fluorescence appears brighter due to removal of the partially transparent gold coating.

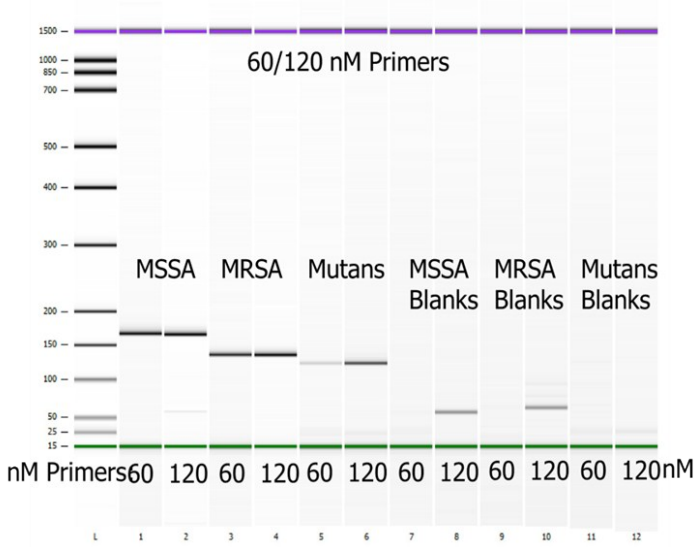

Figure S2. PCR was performed in bulk solutions in tubes using beads to deliver primer concentrations of 60 or 120 nM for MSSA, MRSA, and *S. mutans* sequences. Green and purple bars are the small and large internal control markers.

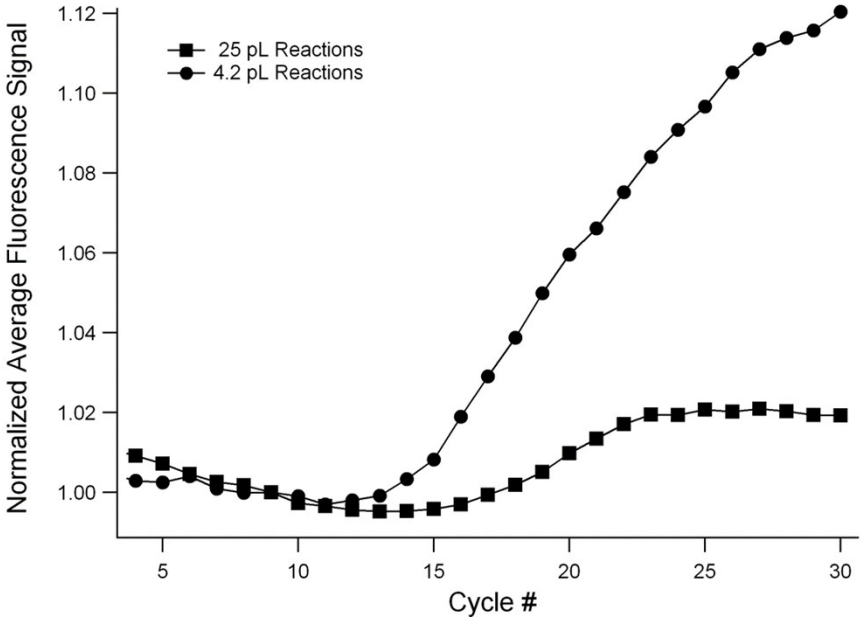

Figure S3. The real-time PCR signal average from several positive wells is shown for 25 pL and 4.2 pL reactions. The higher primer concentration that results with release from a single bead into a 4.2 pL reaction yields better signal to noise than that observed in 25 pL reactions.