Supplementary Materials: Parallel DNA AND Logic Processing with Threshold-Gated Whispering-Gallery-Mode Microcavity Laser Arrays

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S1. Domain-Level Design of the Gates

Each gate is a single complex of a scaffold strand S and a pre-hybridized output strand O (Figure S1a). The scaffold contains four domains, a*, x*, y*, and b*; O covers x* and y* (18 bp), and a* and b* are free toeholds. Input A (x–a) binds a* and displaces the x half of O, and input B (b–y) binds b* and displaces the y half of O (Figure S1b). Each input alone therefore removes only 9 of the 18 bp holding O, whereas both together release it, which is the AND operation. The reporter R (x*–y*) is complementary to O and immobilized on the microsphere (Figure S1c). The full sequences of all strands are listed in Table S1 and the domain sequences of the three channels in Table S2.

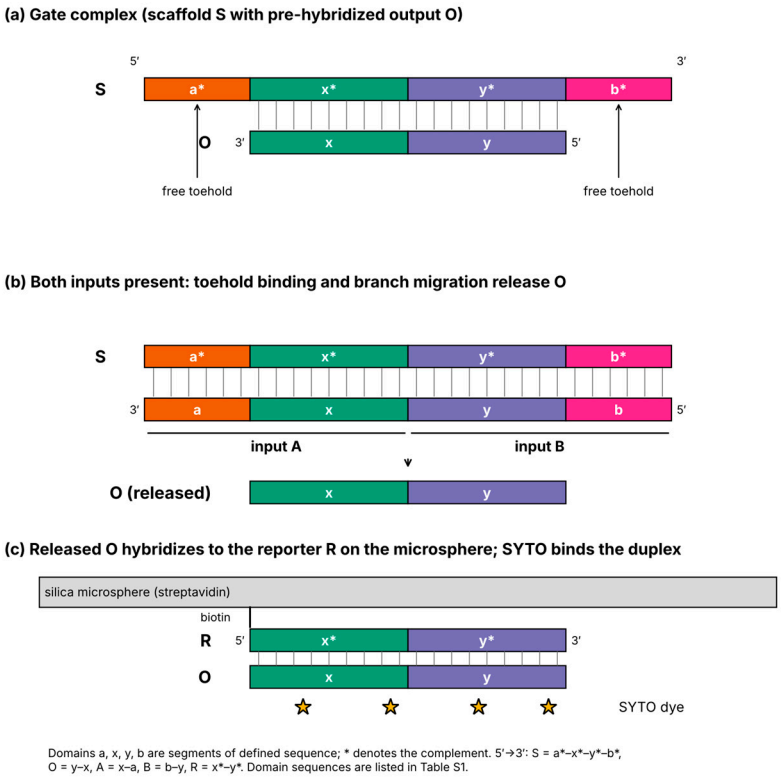


Figure S1. Domain-level design of the AND gates. (a) The gate complex consists of a 30-nt scaffold S and an 18-nt output O hybridized to its central domains x* and y*; the domains a* and b* remain single-stranded as toeholds. (b) Input A binds a* through its 3′ domain a and displaces x of O; input B binds b* through its 5′ domain b and displaces y of O. Only when both are present is O released. (c) The released O hybridizes to the reporter R immobilized on the microsphere, forming a duplex that SYTO dyes bind. The domain structure is identical in all three channels (Table S2).

Table S1. DNA sequences (5′→3′) of the gate scaffold, gate-bound output, input strands, and surface reporter of the three channels. Toeholds are the 6-nt segments at the 3′ end of input A and the 5′ end of input B. The reporter was biotinylated at the 5′ end for immobilization (Section S3).

Component	Sequence (5′→3′)	Length (nt)
Gate scaffold S_1	ACGTTGGCTACGATTCGAGTACCATTCGAC	30
Output O_1 (pre-bound to S_1)	TGGTACTCGAATCGTAGC	18
Input A_1	AATCGTAGCCAACGT	15
Input B_1	GTCGAATGGTACTCG	15
Reporter R_1	GCTACGATTCGAGTACCA	18
Toeholds A_1 / B_1	CAACGT / GTCGAA	6 / 6
Gate scaffold S_2	TGACCACTAGTCCGATGACCTGAAGATCTC	30
Output O_2 (pre-bound to S_2)	TTCAGGTCATCGGACTAG	18
Input A_2	TCGGACTAGTGGTCA	15
Input B_2	GAGATCTTCAGGTCA	15
Reporter R_2	CTAGTCCGATGACCTGAA	18
Toeholds A_2 / B_2	TGGTCA / GAGATC	6 / 6
Gate scaffold S_3	CAGTTCAGCTGATCCGTAGACTTCTGCAGA	30
Output O_3 (pre-bound to S_3)	GAAGTCTACGGATCAGCT	18
Input A_3	GGATCAGCTGAACTG	15
Input B_3	TCTGCAGAAGTCTAC	15
Reporter R_3	AGCTGATCCGTAGACTTC	18
Toeholds A_3 / B_3	GAACTG / TCTGCA	6 / 6

Table S2. Domain sequences (5′→3′) of the three gates. With * denoting the complement, S = a*–x*–y*–b*, O = y–x, A = x–a, B = b–y, and R = x*–y* (5′→3′). The full strand sequences are given in Table S1.

Domain (5′→3′)	Channel 1	Channel 2	Channel 3
a (toehold, 3′ end of A)	CAACGT	TGGTCA	GAACTG
x (9 nt)	AATCGTAGC	TCGGACTAG	GGATCAGCT
y (9 nt)	TGGTACTCG	TTCAGGTCA	GAAGTCTAC
b (toehold, 5′ end of B)	GTCGAA	GAGATC	TCTGCA

S2. Solution-Phase Fluorescence Kinetics

Reaction conditions: To verify AND-gate operation independently of the microsphere laser readout, the reactions were followed in solution without microspheres. Gate complexes (100 nM), free (non-immobilized) reporter strands (100 nM), and SYTO 13 (5 μM) were prepared in Tris–acetate–EDTA (TAE) buffer containing 12.5 mM $MgCl_2$ (TAE/12.5 mM $MgCl_2$), and the input strands (A, B, or both; 100 nM each) were added at t = 0. The reactions were followed at room temperature. For each of the three gates, the

following conditions were measured: gate only (0,0), gate + A (1,0), gate + B (0,1), gate + A + B (1,1), A + B without gate (without the reporter), and the preformed O + R duplex (100 nM) as the fluorescence reference. SYTO 13 was used for all three gates so that all conditions were read in the same spectral window around 520 nm. The gate-free A + B mixture and the single-input conditions test whether direct hybridization between the inputs or single-input leakage contributes to the signal.

Optical readout: Fluorescence was excited and collected with the optical setup used for the laser measurements (OPO pump near 488 nm, 5 ns pulses, 20 Hz; free-space collection). It was read from the emission spectra (integrated over ±10 nm around 520 nm) and is given in arbitrary units, normalized to the preformed O + R signal of the same gate (= 1.0). The pump and the spectrometer exposure (3 s, approximately 60 pulses per spectrum, entrance slit narrowed to avoid detector saturation) were not synchronized. The data are therefore relative; mean ± standard deviation of three repeated measurements is shown.

To verify the molecular AND operation independently of the laser readout, the reaction of each gate was followed in solution by SYTO 13 fluorescence (Figure S2). In all three gates, the (1,1) signal increased monotonically after both inputs were added and approached 0.79, 0.68, and 0.86 of the preformed O + R reference at 60 min for gates 1, 2, and 3, respectively, whereas the gate-only (0,0) and single-input (1,0) and (0,1) conditions remained at 0.07–0.12 throughout. At 60 min, the (1,1) signal was approximately 6–7 times higher than the highest single-input signal. These relative values should not be read as reaction yields, because SYTO fluorescence reports the amount of dye-accessible duplex rather than the concentration of a specific strand.

The gate-free A + B mixture, which tests direct hybridization between the two input strands, started at 0.11–0.12 and rose slowly to approximately 0.15–0.16 at 60 min. This residual signal was about one fifth to one quarter of the gate-mediated (1,1) signal. Because this mixture contained no reporter, it may reflect a weak interaction between the two input strands or dye binding to single-stranded DNA; in either case it is small compared with, but not zero relative to, the gate-mediated response. The (1,1) signal did not reach the preformed O + R reference, which is partly expected because the scaffold was used in slight excess (approximately 1.2:1 over the output strand) to avoid free output strands: the excess scaffold can also bind the input strands without releasing an output. In the laser experiments, unbound DNA was removed by rinsing (Section 2.3 of the main text), so that only duplexes anchored to the surface-immobilized reporters contributed to the readout.

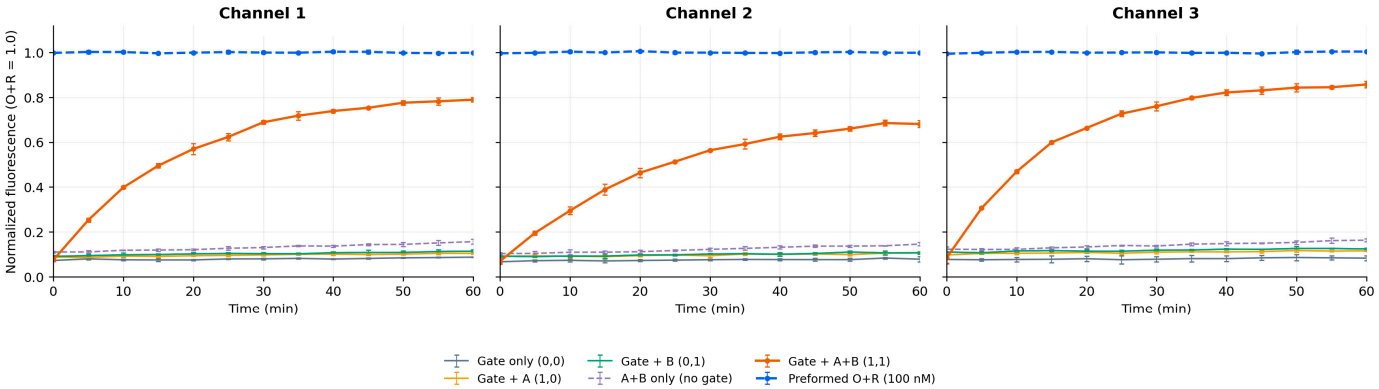


Figure S2. Solution-phase fluorescence kinetics of the three AND gates. All conditions were read with SYTO 13 near 520 nm, so panels are labeled by gate (channel numbers as in Table S1), not by dye. Fluorescence is given in arbitrary units, normalized to the preformed O + R duplex (100 nM) of the same gate (= 1.0). Gate only (0,0), gate + A (1,0), gate + B (0,1), gate + A + B (1,1), A + B without gate, and the preformed O + R reference are shown. Mean ± standard deviation of three repeated measurements.

Control measurements: SYTO 13 alone and SYTO 13 with input A or input B alone (without gate) gave signals at the noise level (more than 20 dB below the preformed O + R reference).

S3. Microsphere Functionalization and Reporter Immobilization

Microspheres: Acid-washed silica microspheres (diameter approximately 100 µm; Benchmark Scientific) were functionalized following our previous study (ref. [18] of the main text).

Silanization: The microspheres were soaked in 5% (3-aminopropyl)trimethoxysilane in methanol for 30 min, rinsed with ethanol, cured at 110 °C for 1 h, and cooled to room temperature.

Crosslinker and streptavidin coating: The surface was treated with 0.1 mg/mL bis(sulfosuccinimidyl)suberate (BS3) in phosphate-buffered saline (PBS) for 30 min. After a PBS rinse, the microspheres were soaked in 1 mg/mL streptavidin in PBS for 30 min and rinsed with PBS, deionized water, and TAE buffer containing 12.5 mM $MgCl_2$.

Reporter immobilization: The 5′-biotinylated reporter strand of each channel (Table S1; 1 µM in TAE/12.5 mM $MgCl_2$) was bound to the streptavidin-coated surface for 30 min. Microspheres carrying different reporters were kept separate and loaded into their respective channels.

Channel loading and surface characteristics: The microspheres were suspended in TAE/12.5 mM $MgCl_2$ buffer and introduced into the PDMS channels. Each channel was fabricated with a recess at the interrogation position, and the glass substrate and PDMS layer were bonded after the recess had been formed, so that microspheres are held in place as the solution flows past them. A recess can hold more than one microsphere, but because the density is low, a single microsphere was located by scanning the chip with a microstage and measured. The positioning of microspheres in the recesses is not reproducible. No additional blocking step was used. The reporter surface density was not determined; the reporter was applied so as to cover the surface as densely as possible, and SYTO dyes were supplied at high concentration for the same reason.