Supplemental Document

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7X multiplexed, optofluidic detection of nucleic acids for antibiotic-resistance bacterial screening: supplement

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Device fabrication

The ARROW chips were fabricated on a 100 mm <100> oriented silicon wafer. A sequence of dielectric layers was sputter deposited on the substrate for optical guiding. These cladding layers consisted of Ta_2O_5 and SiO_2 (refractive index 2.107 and 1.46 respectively). The sequence of the dielectric material layers was chosen to be SiO_2/ Ta_2O_5/ SiO_2/ Ta_2O_5/ SiO_2/ Ta_2O_5/ starting from the substrate with thickness 265/102/265/102/265/102/ nm. SU8 photoresist (Microchem) was spun on top, patterned and developed to define a 5 µm x 12 µm (thickness x width) LC waveguide channel. SU8 photoresist and a thin nickel layer were used as a mask to selectively etch a self-aligned pedestal into the wafer using a reactive inductively coupled-plasma reactive ion etcher (ICP-RIE). A single layer of 5 µm SiO_2 (refractive index 1.51) was deposited on top of the SU8 by plasma-enhanced chemical vapor deposition (PECVD). 4 µm tall ridges were etched into this SiO_2 layer using ICP-RIE to form the single mode SC waveguide, MMI waveguide and SC collection waveguides which intersect the LC waveguide at multiple points. The thickness and etch depth of the waveguide were optimized to tightly confine the modes for proper self-imaging by the MMI waveguide. On top of this, a 6 µm thick protective layer of low index SiO_2 (refractive index 1.448) was deposited using PECVD, thus forming buried ridge SC waveguides. Fluidic inlets into the LC waveguide channels were exposed with a wet etch through the top layers of SiO_2 and the SU8 was then removed with H_2SO_4 : H_2O_2 solution to form the hollow core. The buried architecture prevents water absorption of the waveguides during cleaning steps and helps improve optical coupling between SC and LC waveguides. Fig. S1 shows cross sectional SEM images of both the solid and hollow-core waveguides.

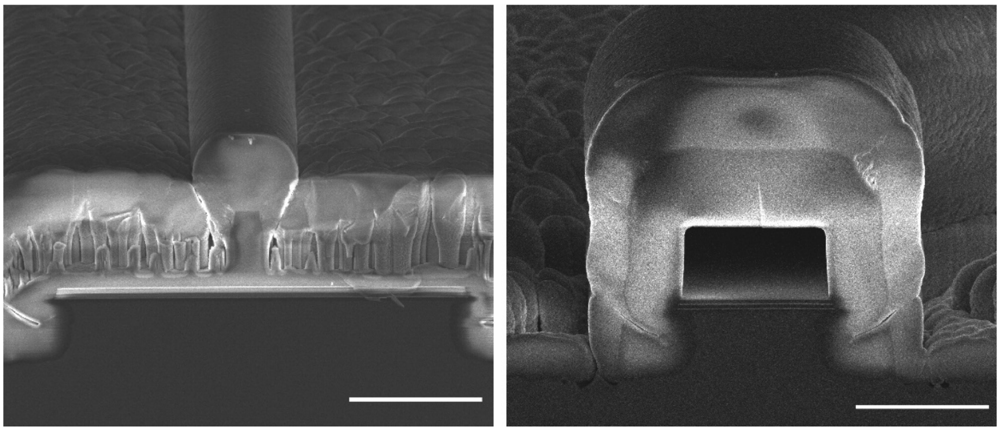

Figure S1: Cross sectional SEM image of SC and LC ARROW waveguides (Scale bar: 10 µm). The SEM image clearly shows the ARROW layers on the Si substrate. The SC waveguide has a high refractive index SiO_2 (1.51) core and low refractive index SiO_2 (1.448) cladding.

Assay preparation

10 µL of 3 µM synthetic nucleic acid strands corresponding to antibiotic resistant bacterial target was mixed with 10 µL of 10 µM of target specific fluorescent probe oligomers [IDT DNA Inc.]. The final volume for each target-probe mixture was made to 30 µL by adding 1XT50 buffer. The target-probe mixture was heated to 95^0 C for 5 mins and incubated for 3 hrs. Streptavidin coated magnetic beads [Dynabeads^{TM}] were functionalized with target specific biotinylated capture oligomers. After incubation the hybridized target-probe structure was mixed with the functionalized magnetic bead and rotator mixed for 1 hr. A magnet kept under the vial was used to pull down the magnetic bead assay with the captured target-probe complex. All unbound nucleic acid strands were washed off and the assay was resuspended.

δt_C Calculation

The total time of an event ΔT is found from the start and end of an event signal using a peak finding algorithm (Fig. S2). The velocity of each assay is calculated from the total time ΔT taken by the assay to travel the 75 µm wide MMI waveguide intersection. δt_c is then calculated by using the distance between adjacent spots (δX_C, Fig. S2) generated by the MMI waveguide for the three excitation wavelengths (Equation S1).

$$\delta t_C = \frac{\Delta T \cdot \delta x_C}{75\mu m} \qquad \text{Equation (S1)}$$

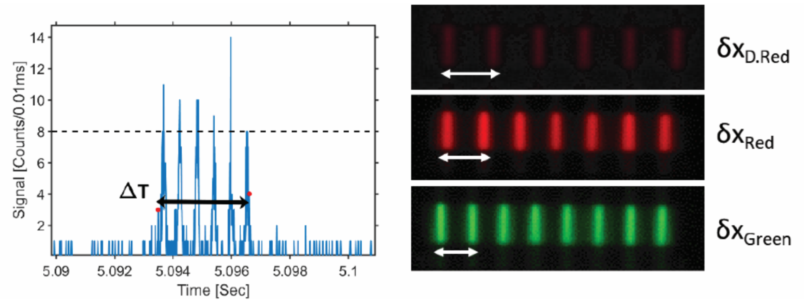

Figure S2 Calculation of δt_c values of individual events

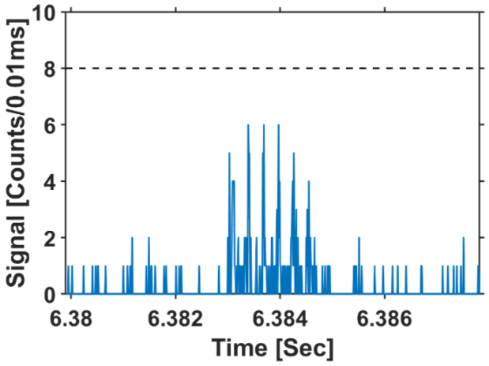

Figure S3 Signal from bead with *E. coli* target below the threshold

Target name	Capture Oligomer	Fluorescent probe oligomer	Color Code
E.Coli	5’AACGGGGAAACTCAGCAAGC GCACTTACAGG/3BioTEG/	5’/5Alex750N/GCT ACCAAGCCGAAAGAACTGTA CAGCGAAGAGGCAGTGTAGC3’	Dark red
KPC	5’GGTGGCAGAAAAGCCAGCCA GCGGCCATGAGAGACAAGAC AGCAGAACTAGACGGCGATA /3BIOTEG/	/5TYE665/CGCACAA AGTCCTGTTCGAGTTTAGCG AATGGTTCCGCGACGATGCG3’	Red
VIM	5’TCTGGTAAAGTCGGACCTCT CCGACCGGAATTTCGTTGAC TGTCG/3BioTEG/	5’TCGCCGTCAAACGACTGCGT TGCGATATGCGACCAAACAC CGGCGA/3AlexF546N/	Green
IMP	5’GATGGATTGAGAATTAAGCC ACTCTATTCCGCCCGTGCTG TCAGCCCG/3BioTEG/	/5Alex750N/TTTAAGCAGCTCATTAGT TAATTCAGACGCATACGTGGG3’ /5TYE665/ACTAGGTTATCTGGAG TGTGTCCTGGGCCTGGAT3’	Dark red-Red
NDM	5’ATGTCGAGATAGGAAGTGTG CTGCGAAACCCGGCCAGACA TTCGGTGCGA/3BioTEG/	/5Alex546N/ACC GACATCCCTGACGATCAAAC CGTTGGAAGCGACTGTCGGT3’ 5’GATGCGTTGATCTCCTGCTT GATCCAGTTGAGGATCTGGG CGCATC/3TYE665/	Red-Green
K.Pneu	/5BiotinTEG/GTCGCCAGGC CGCTGGCGCGCTTGGTCATA AAGTTATCGGTCTGGGCAGA3’	/5Alex546N/ CGCCGCCGAATT CCGGGAACATATCGGTCCAG3’ 5’CGTACAGGGCGCCAAGGTTA CGACCGTAGTC/3AlexF750N/	Green-Dark red
E.Aero	5’AACAACCGTATCGGTGACAA CCATGACACCGGCGTTTCCC CAGTATTCGCTGGCG/3BioTEG/	5’CCGTCTGGGCGGCATGGTAT GGCGCGCTGACTCC /3AlexF750N/ /5Alex546N/CTGGGTTACCCGA TCACTGACGATCTGGACATC /5TYE665/CGGCGCATTCAAAG CTCAGGGCGTTCAGCTGACC	Dark red-Red-Green

Table S1. Oligomer summary

Summary of the oligomers used for fluorescent tagging and capturing of the synthetic target nucleic acids.