# Integration of Nucleic Acid Amplification, Detection, and Melting Curve Analysis for Rapid Genotyping of Antimicrobial Resistance

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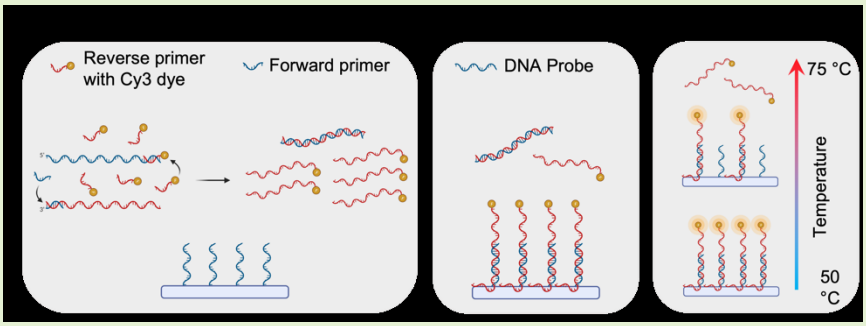
***Abstract*—To prevent the overuse of antibiotics and reduce the spread of antimicrobial resistance (AMR), we developed an integrated genotypic test to identify target AMR genes and mutations with minimal user operation. The integrated AMR sensor consists of a reaction chamber built on a total internal reflection (TIR)-coupled DNA microarray, a temperature management unit, and a compact fluorescence reader. By executing a programmed temperature profile, the automated system can perform asymmetric polymerase chain reactions (PCR) to amplify multiple target genes, microarray hybridizations to detect the amplicons, and melting curve analysis (MCA) to identify resistance mutations. Eight AMR genes selected from *Acinetobacter baumannii*, *Klebsiella pneumonia*, *Escherichia coli*, *Campylobacter coli*, and *Campylobacter jejuni* were amplified using the asymmetric PCR and subsequently detected using the TIR-coupled microarray. The point mutation in the quinolone resistance-determining region of the gyrA gene of *Campylobacter jejuni* was further studied by performing MCA on the TIR-coupled microarray. The benefits of integrated and rapid assay, compact and automated instrument, and multiplexed analysis would facilitate point-of-care antimicrobial susceptibility testing for bacterial infections.**




***Index Terms*—Antimicrobial resistance, DNA microarray, melting curve analysis, point-of-care testing.**

## I. Introduction

OVERUSE and misuse of antibiotics for treatment and prevention of infectious diseases are major contributors to the spread of antimicrobial resistance (AMR), which is a major public health concern and is considered a silent pandemic [1-3]. At present, there are urgent needs for rapid AMR tests that can be used by physicians to rapidly diagnose infections and reduce the excessive use of antibiotics. In a clinical laboratory, antimicrobial susceptibility testing is used to determine whether pathogenic bacteria are resistant to specific antibiotics. The most widely used tests are phenotypic and culture-based techniques, including broth dilution and disk diffusion [4, 5]. These traditional culture-based tests take one to three days to obtain results and become even longer for slow growing pathogens [6]. Point of care tests that allow physicians to diagnose bacterial infections quickly and accurately, and prescribe specific, rather than broad-spectrum antibiotics, are highly desired to strengthen antimicrobial stewardship.

In contrast to the time-consuming process involved in culturing bacteria, nucleic acid tests, including polymerase chain reaction (PCR) tests, DNA microarray assays, CRISPR-based assays, and genome sequencing technologies, are genotypic alternatives to identify AMR profiles [7-11]. To date, an increasing number of AMR genes have been documented in comprehensive antibiotic resistance databases, which enable the implementation of these genotyping approaches. The real-time PCR and isothermal PCR can amplify target genes and provide quantitative results but lack a sufficient throughput to simultaneously detect multiple AMR genes. The genome sequencing-based approaches can detect AMR genes and mutations but require library preparation and robust bioinformatics analysis [12]. DNA microarray can simultaneously measure a large number of genes by hybridizing the target sequences to probe oligonucleotides on a solid substrate, and the results of the hybridization can be quantified using signal reporters [13-15]. To date, DNA

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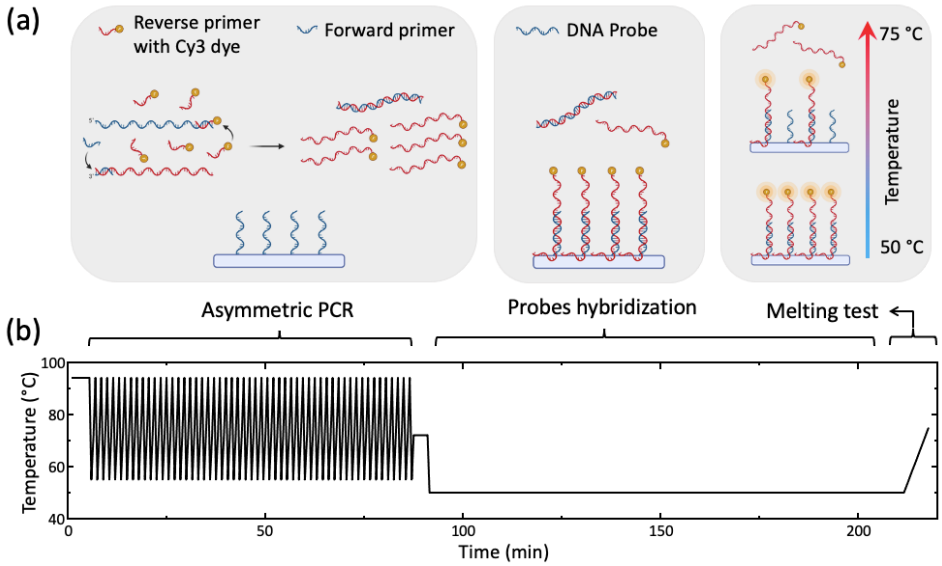


Fig. 1. **Integrated amplification, detection, and melting analysis of AMR genes.** (a) Schematic of the AMR gene analyzing assay that consists of three consecutive steps: asymmetric PCR amplification (left), microarray hybridization (center), and MCA (right). (b) Temperature profile programmed to run the integrated assay for a total period of 3.75 hrs.

microarrays have been successfully implemented to detect disease-related genes, as well as identify pathogenic infections [16-18].

This paper reports an automated AMR gene sensor in conjunction with an integrated assay for the amplification, detection, and mutation analysis-all in a single chamber, to determine the AMR genes and mutations. The AMR gene sensor combined the advantages provided by three different DNA analysis techniques, while offsetting the drawbacks of each to simplify the genotypic AMR testing. The proposed system consisted of a single reaction chamber built upon a DNA microarray, a temperature management unit, and a compact fluorescence reader. As illustrated in Fig. 1(a), multiple target genes can be amplified in the reaction chamber and measured using the DNA microarray. Then, an on-chip melting curve analysis (MCA) was performed to identify mutations [19-23]. In order to eliminate the background fluorescence signals, the microarray was excited using a total internal reflection (TIR) scheme, which coupled a laser excitation into the microarray substrate and used its evanescent field only to excite the fluorophores attached to the DNA microarray [24, 25]. To run a test, users only need to introduce genomic DNA samples and reagents into the reaction chamber and wash the reaction chamber before the microarray imaging step. A microcontroller was programed to synchronize and execute most assay steps by setting the chamber temperature (Fig. 1(b)), acquiring fluorescence images, and analyzing the results. Here, as a proof of concept, the system was used to identify seven AMR genes and one resistance-conferring mutation from five different pathogenic species.

## II. Methods and Materials

### A. Materials and Reagents

All oligonucleotides were purchased from Integrated DNA Technologies (Coralville, USA). The DNA microarray was fabricated by Daicel Arbor Biosciences using digital photolithography and phosphoramidite oligonucleotide synthesis chemistry. The multiplex PCR kit (M0284S) was obtained from New England Biolabs Inc.. All primers were obtained from Integrated DNA Technologies. The phosphate-buffered saline (PBS), and agarose gel (G700802), and bacterial DNA purification kit (A29790) were purchased from ThermoFisher Scientific.

### B. Automated AMR Sensor Chip

The oligonucleotide microarrays were printed on

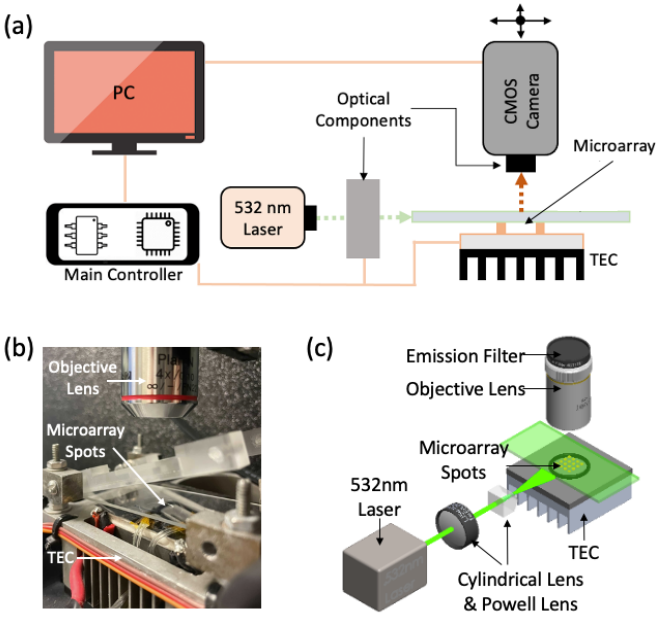


Fig. 2. **Instrument assembled to perform the integrated AMR analysis.** (a) Schematic diagram of the thermal management system, fluorescence detection unit, excitation beam sub-assembly, optical components, and the microcontroller. The Arduino microcontroller is used to interface with all sub-units for the temperature management and acquisition of fluorescence images. (b) Photos of the microarray, reaction chamber, and TEC controller. (c) Schematic diagram of the optics for the TIR coupling and fluorescence imaging.

1-mm-thick glass slides. The input edge of the glass slides was polished to reduce light scattering and improve the coupling efficiency. As shown in Fig. 2(a), a 10-mm-diameter silicone gasket chamber was mounted onto the microarray with the DNA probes facing towards the chamber. Fluorescence emissions from the microarray were coupled out of the reaction chamber through the glass substrate. The bottom side of the reaction chamber was attached to a thermoelectric cooler (TEC). An acrylic frame was machined to press the microarray slide and reaction chamber against the TEC (Fig. 2(b)). A k-type thermocouple (DP25B-TC, OMEGA) was inserted inside the reaction chamber to monitor reagent temperature. The TEC and thermocouple can set the reaction temperature based on the temperature profile programmed for the PCR, probe-target hybridization, and denature conditions. The glass slide can be detached from the silicone chamber to wash the chamber before imaging.

An Arduino Uno microcontroller unit (MCU) was programmed to automate the assay by performing the DNA amplification, hybridization, and melting steps summarized in Fig. 1. The MCU can set chamber temperature precisely using the TEC and thermocouple to generate and dissipate heat during heating and cooling stages. According to the test protocol, a target temperature profile was uploaded to the MCU and executed using an adaptive proportional-integral-derivative feedback loop. Chamber temperature was measured every 0.5 s and stored in the MCU. To acquire fluorescent images, the MCU sent trigger signals to the laser as well as the CMOS imaging sensor. The CMOS camera can transfer acquired images to a desktop PC for image processing.

Table I
LIST OF TARGET GENES, ANTIBIOTIC RESISTANCE, MICROORGANISM, PCR PRIMERS, AND MICROARRAY PROBE SEQUENCES

| Gene | Resistance | Microorganism | Primer sequence (5'→3') | Probe sequence |
|---|---|---|---|---|
| blaNDM-1 | β-*lactam* | *A. baumannii* | (*F*) GCGCAACACAGCCTGACTTT<br>(*R*) CAGCCACCAAAAGCGATGTC | GCAGCACACTTCCTATCTCGACATG |
| blaOXA-23 | β-*lactam* | *A. baumannii* | (*F*) TTCTGGTTGTACGGTTCAGCA<br>(*R*) TGCCCAACCAGTCTTTCCAA | GGAGAACCAGAAAACGGATATTAATGAAAT |
| cfr(C) | *Linezolid* | *C. coli* | (*F*) GGTGAAACTGTTGTGGAGAT<br>(*R*) AGTTTCCGTAACTGTCGTTT | GTCCAAGCGACGCATCACCATTTCG |
| gyrA | *Fluoroquinolone* | *C. jejuni* | (*F*) TGCTCTGCTTTTGTGAATTA<br>(*R*) AAACTGCTGTATCTCCATGT | ACCCACATGGAGATACAGCAGTTTATGATGC |
| blaKPC-3 | Ceftazidime/ avibactam | *E. coli* | (*F*) TCCGGTTTTGTCTCCGACTG<br>(*R*) CGGTGTGTACGCGATGGATA | GTTGCTGAAGGAGTTGGGCGGCC |
| blaVIM-1 | β-*lactam* | *K. pneumoniae* | (*F*) CAAGTCCGTTAGCCCATTCC<br>(*R*) GGCACAACCACCGTATAGCAC | GGTGATGAGTTGCTTTTGATTGATACAGCGT GGGGT |
| blaTEM-1 | β-*lactam* | *K. pneumoniae* | (*F*) TTGTTGCCGGGAAGCTAGAG<br>(*R*) TCCTTGAGAGTTTTCGCCCC | GGTTACATCGAACTGGATCTCAA |
| blaOXA-181 | *Carbapenems* | *K. pneumoniae* | (*F*) AGCTTGATCGCCCTCGATTT<br>(*R*) GCTCAGAAACGTGCAGCTTG | ACATAAATCACAGGGCGTAGTTGT |

### C. TIR-based Fluorescence Imaging Setup

The optical detection setup is schematically shown in Fig. 2(c). To achieve a high signal-to-noise ratio, the TIR configuration was adopted to measure the fluorescent images of the microarrays. The excitation light from a solid-state laser ($\lambda$ = 532 nm, $P_{max}$ = 250 mW; Civil Laser Supplier) was coupled into the glass substrate from a polished edge. The laser beam was shaped into a 1 × 20 mm rectangle using a lens assembly, including a Powell lens (43-473, Edmund Optics) and a cylindrical lens (LJ5440RM, Thorlabs). Approximately, 50% of the laser light was coupled into the glass substrate by its edge, traveled along the substrate across the microarray pattern, and exited from the opposite edge. The evanescent field decayed exponentially into the solution above the microarray and only excited fluorophores on the surface of the glass substrate. As a result, the TIR configuration can eliminate the background emission from fluorophores in the solution and enable the melting curve analysis. Fluorescent images of the microarray were collected using a 4× objective lens (PLN4X, Olympus), filtered by an emission filter (MF497-16, Thorlabs), and recorded using a monochromatic CMOS sensor (ASI1600MM, ZWO). To avoid photobleaching of fluorophores, the laser was only turned on when fluorescence images were recorded.

### D. Design of DNA Microarray

The oligonucleotide microarray was fabricated on a glass substrate by Daicel Arbor Biosciences using the light-directed phosphoramidite oligonucleotide synthesis approach [26]. Before the synthesis, the glass slide was silanized and coupled with one 3'-NPPOC protected phosphoramidite base layer. A maskless array synthesizer instrument generated the designed microarray by synchronizing a digital micromirror device with a synchronized chemical delivery system. Each microarray consisted of 153 replicas of a target gene panel and can be used for six tests. The panel included 4 × 4 spots of oligonucleotide probes, as well as positive and negative controls. With the spot diameter of 75 μm and pitch size of 110 $\mu$m, the dimension of each sub-array was approximately 0.4 × 0.4 mm$^2$. The probe sequences were designed for seven popular acquired AMR genes and one gene with mutation conferring resistance from five different bacteria. The probe sequences, corresponding AMR genes, and strains are listed in Table I. The Cy-3 labeled deoxycytidine triphosphate and a sequence that was not present in the sample were used as the positive and negative controls spots, respectively. The printed microarray was blocked using acetylated-bovine serum albumin to prevent non-specific absorptions.

### E. Preparation of Genomic DNAs and PCR Reagent

The genomic DNAs used in this study were extracted from five bacterial species, including *Acinetobacter baumannii*, *Klebsiella pneumonia*, *Escherichia coli*, *Campylobacter coli,* and *Campylobacter jejuni*. The cultured bacteria were lysed, and their genomic DNAs were extracted using Wizard® Genomic DNA Purification Kit (Promega, USA.). The concentration of each DNA sample was determined by a spectrophotometer (NanoDrop, ThermoFisher Scientific). The PCR reagents were prepared by mixing the 25-$\mu$L PCR master mix, 5-$\mu$L primers, 2-$\mu$L purified DNA samples, and 18-$\mu$L PBS. For multiplexed amplification, eight pairs of primers and genomic DNA samples were mixed. For each gene, the concentrations of forward and Cy3-labeled reverse primers were 0.1 and 0.5 μM, respectively. The primer sequences, listed in Table I, were manually designed with a similar melting temperature and minimal crosstalk. To confirm the amplification results, the amplicons were characterized using gel electrophoresis as well as the fluorescence microarray. The DNA gels were prepared using 1.5% (w/v) agarose in gel running buffer with 0.5-μg/ml ethidium bromide. For the DNA gel electrophoresis analysis, 5 μl amplicons were loaded into the gel as well as a 1-Kb DNA ladder. After running the

electrophoresis for 30 min, the gels were imaged using a gel imager (ChemiDoc XRS, Bio-Rad).

## III. Results

The integrated AMR gene analysis assay uses purified genomic DNA samples and aims to detect multiple AMR genes and mutations with minimal user intervention. To achieve the goal, we combined the PCR amplification and DNA microarray assays in one reaction chamber and used the temperature controller to execute the reactions. Fig. 1(a) summarizes the sequential assay steps that include the multiplexed amplification, hybridization, and melting curve analysis. The corresponding temperature profile for these steps is shown in Fig. 1(b). During the 3.75-hours assay, users are only required to initiate a test by introducing the mixture of an extracted DNA sample and the PCR reagents, and then washing the reaction chamber after the DNA hybridization step.

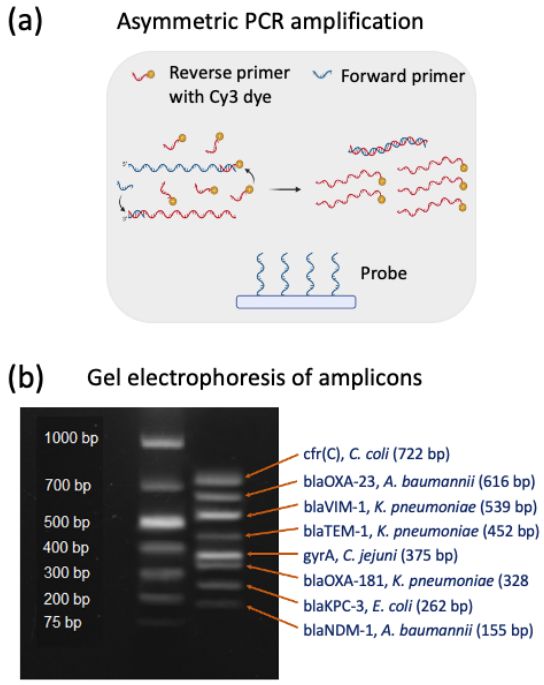


Fig. 3. **Multiplexed DNA amplification using asymmetric primer pairs.** (a) Schematic diagram of the asymmetric PCR amplification performed above the DNA microarray. (b) DNA gel electrophoresis image for the products of the multiplex PCR amplifications. The left Lane: 1Kb DNA marker.

To start a test, 50-µl of DNA sample and PCR reagent were mixed and pipetted into the reaction chamber and the chamber was subsequently closed. The Arduino controller ran 55 thermocycles for asymmetric PCR amplification that includes both exponential and linear amplifications. The asymmetric PCR amplification produced excessive single-stranded DNA (ssDNA) amplicons that were complementary to the probe sequences and facilitated the DNA hybridization. Different concentrations of forward primers (1×) and Cy3-labeled reverse primers (5×) were used, as illustrated in Fig. 3(a). After the forward primers were depleted, the PCR reaction became linear amplification that elongated Cy3-labeled reverse primers to produce Cy3-labeled ssDNA strands. Following the PCR thermocycling, the chamber temperature was set to 50 °C to allow ssDNA molecules to hybridize to their complementary probe sequences printed on the DNA microarray. After a 2-hr incubation, the reaction chamber was washed and refilled using PBS buffer. The microarray was measured using the TIR-coupled fluorescence imager to detect Cy3-labeled amplicons. Finally, the melting test was performed to detect the AMR mutations using the melting curve analysis. The melting curve analysis relied on the TIR-coupled fluorescence detector to record fluorescence images of the DNA microarray during the dissociation of the ssDNAs from the probes. The fluorescence intensity from each spot of interested was plotted as a function of temperature to calculate the melting temperature, which was used to detect single-nucleotide polymorphisms (SNPs) using the difference between the measured curves of the wild-type (WT) and mutation-type (MT) probes [27, 28].

### A. Asymmetric PCRs for Multiple AMR Genes

The performance of the thermocycler sub-system and asymmetric PCR assay were tested by amplifying multiple AMR genes inside the reaction chamber shown in Fig. 2(b). Table I lists the target AMR genes, corresponding bacteria, primers, and the amplicon lengths ranging from 155 bp to 722 bp that used to characterize the proposed system. The genomic DNAs extracted from *Acinetobacter baumannii, Klebsiella pneumonia, Escherichia coli, Campylobacter coli, and Campylobacter jejuni* were mixed. The genomic DNA concentration was approximately $10^4$ copies/µL for each type of bacteria. To confirm the successful amplification of all genes, genomic samples were mixed with the primer pairs and PCR reagent and pipetted into the reaction chamber to be amplified. The PCR reactions were initiated with a pre-denaturing step for 1 min and followed by 55 thermocycles of denaturing (95 °C for 30 sec), annealing (55 °C for 30 sec), and extension (72 °C for 30 sec). The asymmetric PCR assay requires extra amplification cycles to produce Cy3-labeled ssDNA molecules during the linear amplification stage. After the multiplexed PCR amplification, the amplicons were sampled, and all eight amplicons were measured using DNA gel electrophoresis. The image of the gel electrophoresis is shown in Fig. 3(b), where eight clear bands correspond to the target genes with the reference 1Kb DNA marker. The results confirmed the performance of the thermocycler sub-system and the multiplexed PCR assay.

### B. TIR Imaging of DNA Microarray and Detection of Acquired AMR Genes

After the multiplexed amplification of target genes, the Cy3-labeled ssDNAs were hybridized to their corresponding probes printed on the microarray (Fig. 4(a)). During the hybridization, the chamber temperature was maintained at 50 °C for 2 hours (Fig. 4(b)). The binding of Cy3-ssDNA molecules to the DNA microarray spots increased the fluorescence emission of the spots. After the incubation, the reaction chamber was washed and refilled using PBS to reduce

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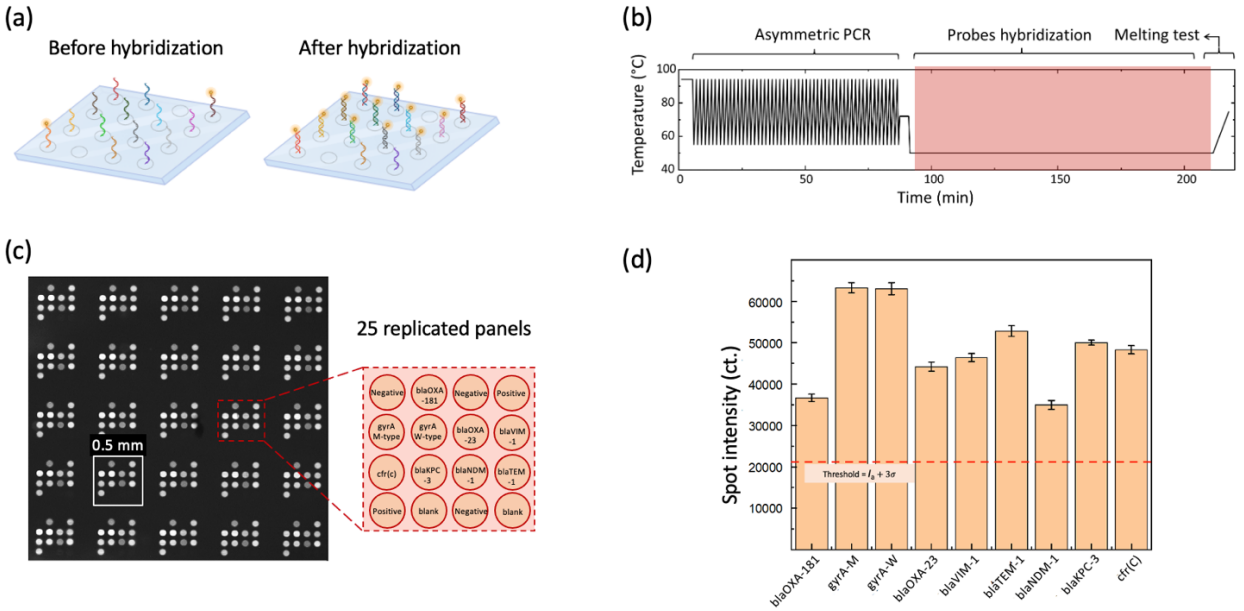


Fig. 4. **TIR image of amplified DNA microarray.** (a) Schematic diagram of the microarray hybridization. (b) Measured chamber temperature with the DNA microarray hybridization step highlighted. (c) Recorded fluorescence image of the hybridized microarray showing a strong fluorescence emission after two hours of hybridization at 50 °C. The negative control spots confirm no outside contamination. (d) Fluorescence intensities mean values of DNA spots for each target gene after a hybridization. The average intensity ($I_a$) plus three times the standard deviation ($\sigma$) of negative control spots was used to calculate a threshold value (dash line) to distinguish positive and negative events.

background fluorescence induced by the unbounded Cy3-ssDNAs. Then, the microarray was measured using the TIR-based fluorescence reader. The 532-nm excitation laser beam was coupled into the glass substrate and guided through the microarray area. The evanescent field of the TIR mode can excite Cy3 dyes on the DNA microarray. Fluorescence images of the microarray were measured using the CMOS imaging sensor with an integration time of 200 ms. Fig. 4(c) shows the measured 16-bit grayscale image of microarray spots after 2 hours of hybridization. The inset represents the layout of the microarray pattern with the positive and negative controls spots and eight target genes. The acquired fluorescence image included 25 replicas of the AMR gene panel (400 µm × 400 µm). For each gene, the average fluorescence intensity was calculated and compared in Fig. 4(d). A threshold, represented by the dashed line in Fig. 4(d), was calculated based on the negative control spots and used to distinguish positive and negative results. It can be seen that the multiplex PCR step can amplify all target genes, and the DNA microarray can subsequently quantify these amplicons, demonstrating a high efficiency of the TIR-based detection of acquired AMR genes.

### *C. Microarray MCA to Detect Point Mutations Conferring Antimicrobial Resistance*

In addition to acquiring AMR by horizontal gene transfer, microbes can also develop AMR due to mutations [29]. The regular microarray hybridization analysis can detect the presence of AMR genes but lacks sensitivity to identify nucleotide mutations. Several approaches have been recently demonstrated for the mutation analysis [30, 31]. Among them, the MCA assay, which assesses the dissociation characteristics of double-stranded DNA (dsDNA) during heating, can be sufficiently sensitive to detect SNPs. Here, the

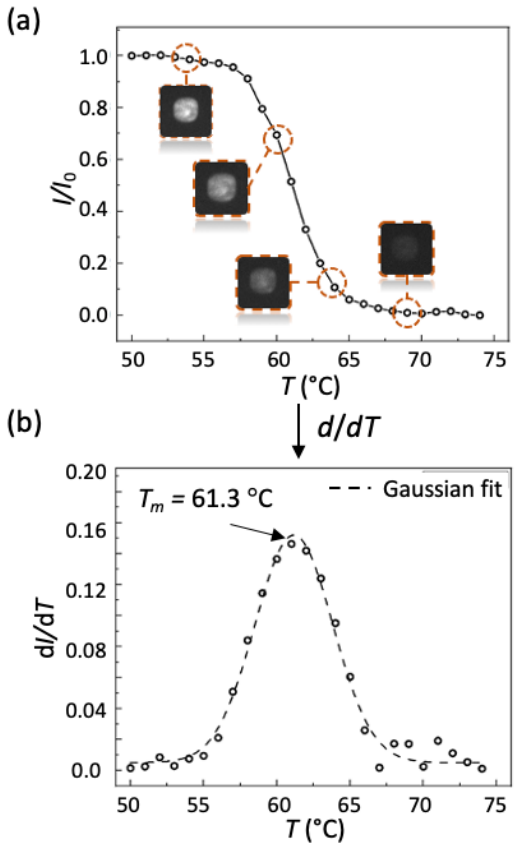


Fig. 5. **Measured melting curves of *cfr(C)* gene.** (a) Normalized intensity as a function of temperature from the cfr(C) spots. Insets: fluorescence images of a cfr(C) spot captured at 55 °C, 60 °C, 63 °C and 68 °C, respectively. (b) Derivative of the melting curve to show the peak that represents $T_m$. The derivative curve was fitted using the Gaussian distribution function to calculate the $T_m$ value.

MCA was implemented to measure the change of Cy3 emission during the dissociation of previously hybridized dsDNAs as the chamber temperature rose. The spot emission intensity decreased when dsDNAs dissociated and the temperature when 50% of dsDNAs were denatured was calculated as the melting temperature. It is possible to distinguish between wild-type and mutant sequences based on the pattern of melting temperatures.

After the TIR imaging of a hybridized microarray, the melting test started by heating up the chamber from 50 °C to 75 °C with a ramp rate of 4 °C/min. In the meantime, the TIR fluorescence images and chamber temperature were recorded every 62.5 ms with an approximated temperature interval of 0.25 °C, until the chamber temperature reached the final temperature of 75 °C. Acquired images were analyzed using a Matlab script to identify spots and find their fluorescence intensity, as well as the corresponding temperate point. For each spot, average fluorescence intensity was calculated and plotted as a function of chamber temperature. Here, we first used cfr(C) as an example to elaborate the MCA process. Since fluorescence emission of the Cy3 dye decreases with the increase of temperature, the melting curves ($I(T)$) were generated by plotting the normalized fluorescence intensity versus temperature as shown in Fig. 5(a). For a given targe-probe pair, the melting temperature can be found by plotting the derivative of the melting curve (Fig. 5(b)). The peak in the $dI(T)/dT$ plot represents the melting temperature. The melting temperature of the cfr(C) gene was found at 61.3 °C, which is close to the theoretical melting temperature of 61.2 °C. The main advantage of using the DNA microarray is simultaneously running multiple melting tests of different dsDNA sequences in a single test.

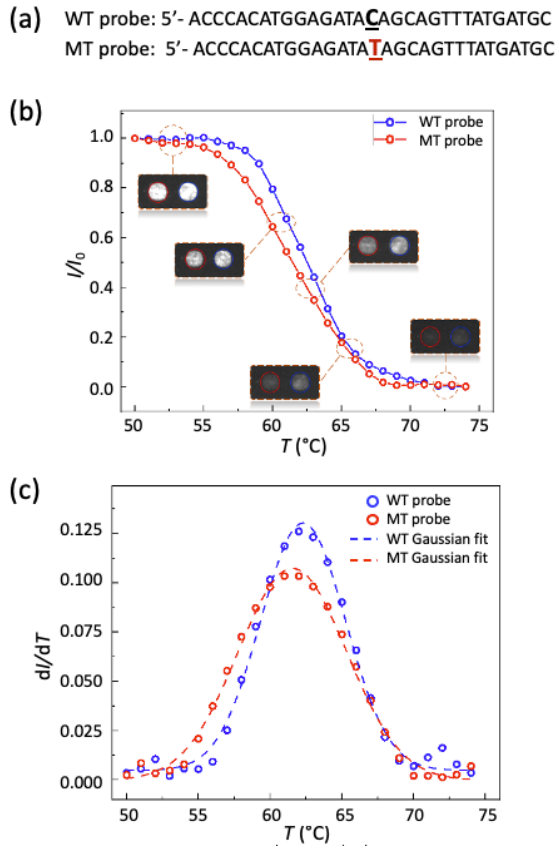


Fig. 6. **MCA of *gyrA* genes from the wild-type and mutant strains.** (a) Calculated melting curves of the WT *gyrA* amplicon to the WT and MT probes. Inset: fluorescence images of both WT and MT spots at five different temperatures. (b) Derivative melting curves of the WT and MT spots. The peaks correspond to the $T_m$ values of 61.5 °C and 60 °C for the WT and MT samples, respectively.

Fig. 6 compares the melting curves of *gyrA* genes extracted from the wild-type (WT) *Campylobacter jejuni* strain and measured using the WT and mutant (MT) ssDNA probes. As highlighted in Fig. 6(a), The MT gyrA probe sequence differs from the WT probe sequence by a single nucleotide, which was mutated from cytosine to thymine. It can be seen from Fig. 6(b) that the WT and MT spots' fluorescence signals started to decay at different temperatures. The shift of the melting curves showed up as a different peak in the derivative plot of the WT and MT melting curves (Fig. 6(c)). Based on the derivative melting curves, the $T_m$ values for the WT and MT spots were calculated as 61.5 °C and 60 °C, respectively. The results show that the melting curve analysis has the resolution to distinguish the single-stranded WT *gyrA* amplicon from the WT and MT probes. When the DNA microarray probes both MT and WT gyrA amplicons at the same time, the melting temperature of hybridized gyrA spots will deviate from indicating the presence of mutations conferring antimicrobial resistance.

## IV. Conclusion

This work successfully demonstrated the AMR gene profiler using the TIR-coupled DNA microarray and the automated nucleic acid analysis assay. The assay, designed with a task sequence of DNA amplification, microarray hybridization, and MCA, can be performed by simply setting reaction temperature. The Arduino microcontroller was used to synchronize with the microarray imaging and reaction temperature setting. The asymmetric PCR increased the target ssDNA concentrations and facilitated the target-probe hybridization on the DNA microarray. The implementation of the TIR excitation limited the fluorescent detection to the microarray surface and effectively avoided the background fluorescent emission from the dissociated Cy3-labeled ssDNA oligos. The results showed that the system could identify multiple AMR genes and mutations from different pathogenic bacterial strains. The conventional cell-based approaches require one to two days to characterize the drug resistance of an infection. In contrast, the on-chip AMR gene profiler has the advantage of multiplexed detection, short detection time, inexpensive instrument, and minimal user operation.

The AMR gene analyzing technology can be improved from the following aspects. First, the assay can be further simplified by eliminating the washing step after the microarray hybridization step. Nanopatterned substrates, such as photonic crystal or plasmonic structures, can be adopted to enhance the fluorescence emission by their spatially confined optical resonances [32, 33]. Used as the DNA microarray substrate, these substrates will offer an even higher signal-to-noise ratio to the detection of hybridized amplicons without washing the chamber. Additional amplification can be also achieved using

oligonucleotide barcodes [34]. Alternatively, by labeling the probe DNAs with another fluorescence dye, it is possible to implement Förster resonance energy transfer to distinguish target DNAs bound on the microarray or floating in the solution. Second, the assay condition can be optimized by reducing the PCR cycles and hybridization time to speed up the assay. Third, a more compact fluorescence reader will be designed for a portable instrument that can facilitate field tests. In addition, we will expand the DNA microarray to include more AMR genes and validate the technology using clinic samples. Last but not least, a compact and automated nucleic extraction component, which can lyse cells, extract and enrich genomic DNAs, will be incorporated into the system, for field applications. Once fully developed, the AMR gene analyzer will become a point-of-care diagnostic apparatus for rapid and specific detection of pathogens to benefit the clinical diagnosis and treatment of bacterial infections.

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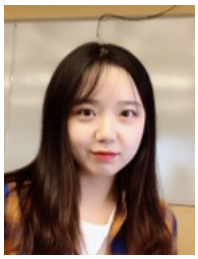

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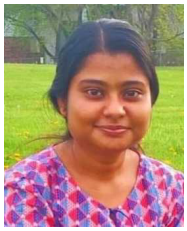

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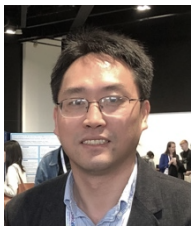

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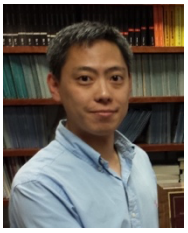

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