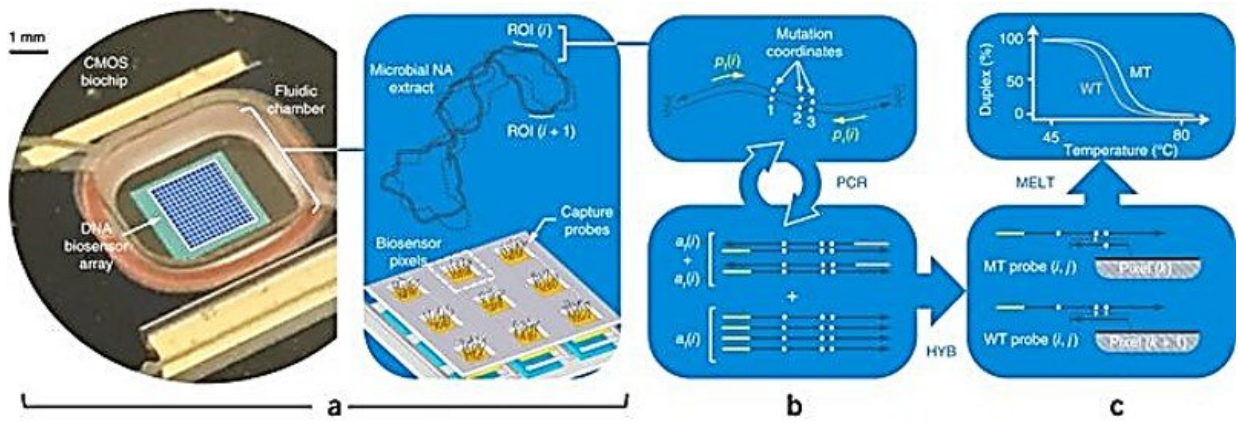
A miniaturized semiconductor biochip to identify drug-resistant pathogens

July 31 2018, by Thamarasee Jeewandara



The NAAT platform architecture; a) the closed tube system contains a fluid chamber affixed to a DNA biosensor array, the chamber can receive microbial nucleic acid (NA) extracts and multiplex polymerase chain reaction (PCR) reagents for solution-phase amplification. The DNA capture probes in close affinity to the amplified regions of interest (ROI) in the microbial genome will functionalize individual biosensor pixels in the system, b) each ROI is amplified via PCR to generate amplicons, c) the amplicons bind to specific capture probes on pixels during real-time hybridization (HYB). Thermodynamic stability of these associations is tested with melting curve analysis (MCA) to differentiate between the wild-type and mutant strain signature. Credit: *Nature Biotechnology*, doi:10.1038/nbt.4179

Evolving strains of multi-drug resistant pathogens are a growing global concern, outpacing drug discovery efforts and undermining the efficacy

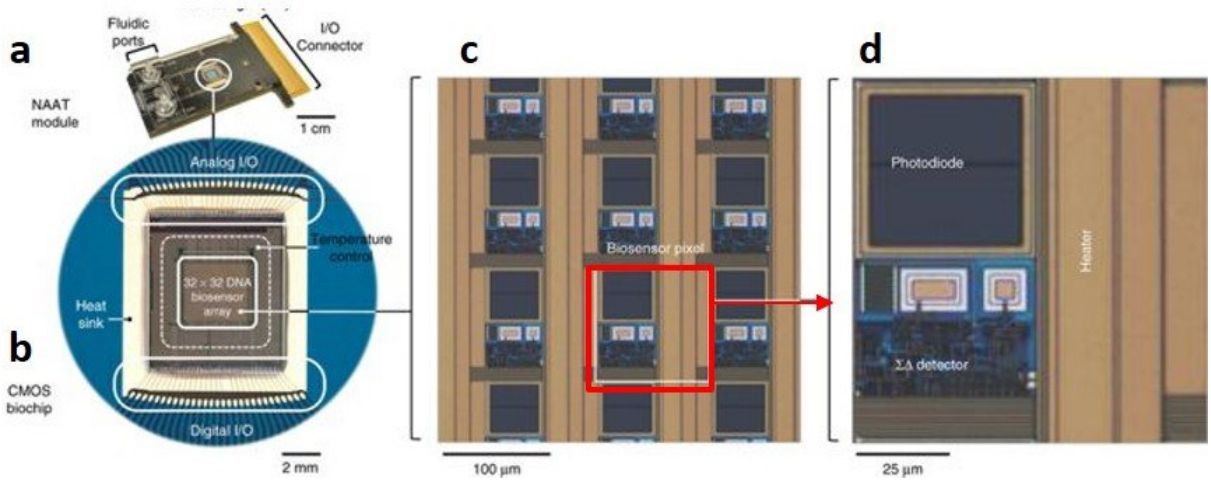
of existing antibiotics. The development of comprehensive diagnostics for clinical applications will become crucial to control escalating health risks. Existing laboratory tests to diagnose infectious disease are generally carried out via culture-based methods that usually take days to generate results. Rapid molecular diagnostic tests can comparatively identify microbial nucleic acids (NA) in clinical samples directly in less than an hour with nucleic acid amplification tests (NAATs). Existing NAATs are, however, limited by inadequate levels of multiplexing (i.e. the number of strains or sequences detected in a single reaction) and inaccuracies with detecting mutations.

Scientists and engineers recently collaborated to develop a fully integrated, miniaturized semiconductor biochip with closed-tube detection chemistry to perform multiplexed NAAT with aims to overcome existing limitations. The system can rapidly amplify DNA and RNA sequences in a single sample, including species signatures, genotypes and mutations that confer pathogenic drug resistance. Feasibility of the system was first demonstrated by identifying and quantifying multiple viral RNAs and DNAs from respiratory tract pathogens. Subsequently, the system was used to detect > 50 antibiotic resistant mutations across multiple genes of the Mycobacterium tuberculosis (MTB) genome. The research is published in *Nature Biotechnology*.

In the study, Hassibi et al. designed and developed a miniaturized and single-use (disposable) semiconductor, using conventional sub-micron, complementary metal oxide semiconductor processors (CMOS). The architecture of the biochip contains a fluid chamber with thermocycling capabilities and DNA capture probes with fluorophores that sit above an embedded biosensor array. The fluid chamber of the system can receive multiplex PCR reagents and microbial nucleic acid of a clinical pathogen – with a specific region of interest responsible for drug resistance—for its amplification. When the amplified region of the microbial genome is

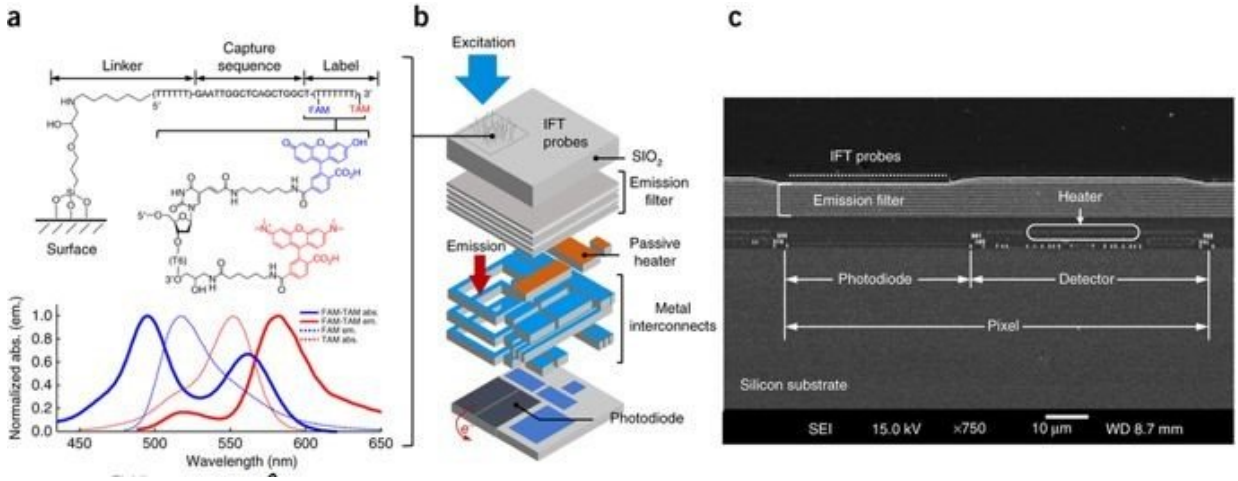
in close affinity to the DNA capture probe on the biochip, individual biosensor pixels will be activated to detect and differentiate between the wild type and mutant microbial strains.

In the NAAT module, fluidic ports can introduce the multiplex PCR mix and extracted nucleic acids into the chamber for nucleic acid amplification. The biochip houses an array of 32 x 32 biosensing pixels, each with embedded photodiode, heaters and detectors. The system is built to identify multiple and distinct nucleic acid sequences in real-time via the inverse fluorescence transduction (IFT) method. By function, the CMOS biochip can detect real-time IFT signals while controlling the temperature of the fluid chamber to enable nucleic acid amplification.



Structure of the biochip and inbuilt components: a) the disposable NAAT module includes a fluidic cap with inlet and outlet ports to b) encapsulate the CMOS biochip mounted on the printed circuit board, c) the biosensor pixels (red highlight) are arranged in a 32 x 32 (1024) array, d) each biosensing pixel is housed with a photodiode, heaters and detectors. Credit: *Nature Biotechnology* doi: doi:10.1038/nbt.4179

Instead of conventional PCR dyes, the system includes real-time transduction with oligonucleotide IFT probes affixed to each pixel, in addition to the individual photodetector and electronic readout circuits housed within to acquire and digitize fluorescent emissions. Each probe constitutes a dual-fluorophore label to hybridize with PCR amplicon analytes. Overall, the sensor array has an adjustable speed of one to 50 reads per second.



Structure of the biosensor pixels: a) the IFT probes contain a modular linker and a dual-fluorophore label (FAM-TAM) to recognize the PCR amplicon analytes, b) schematic illustration of the 3D stack of the biosensor pixel with localized IFT probes, and b) a scanning electron microscope derived cross-section with emission filters, heater, photodiode and detector of a single pixel fabricated on silicon. Credit: *Nature Biotechnology* doi: doi:10.1038/nbt.4179

The platform can detect hundreds of different sequences, only limited by the number of pixels on the array. To demonstrate multiplexing capacity, researchers used the CMOS biochip to simultaneously identify multiple DNA and RNA respiratory viruses in the same clinical sample. The

platform then identified 54 single nucleotide polymorphism mutations (SNPs), located in six genes to detect drug-resistance-conferring mutations, separating the wild type strains (pathogens sensitive to drugs) from the drug-resistant strains.

Although the system has advantages beyond the existing NAAT platforms to include cost-effective multiplexing, workflow simplicity and