RECOMBINEASE POLYMERASE AMPLIFICATION IN RING RESONATORS FOR REAL-TIME AND LABEL-FREE DETECTION OF dsDNA

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Methodology

SURFACE ACTIVATION AND FUNCTIONALISATION

The surface of the chip is activated by the formation of an homogeneous self-assembled monolayer of azidosilane achieved by vapor phase deposition. The rings are then functionalised via a fast “click” reaction using a spotter for functionalisation of specific ring resonators with hexynyl terminated DNA sequences (either forward primers for RPA or complementary strands for direct detection).

SURFACE CHEMISTRY OPTIMISATION

The DNA probe density, spacer length and the backfiller-to-probe ratio was optimised to enhance hybridisation efficiency.

DIRECT DETECTION AND ENZYMATIC DNA AMPLIFICATION

The calibration curve of the ring resonators for the direct detection of ssDNA span five orders of magnitude, with a limit of detection (LOD) of 20 nM. Recombinase polymerase probes were used in order amplify target ssDNA in solid phase by amplification of immobilised forward primers on the chip at constant temperature, yielding an LOD of 7.8·10⁻² M.

CALIBRATION CURVE

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Results

Dna-to-backfiller molar ratios

Conclusion

The use of microring resonators allowed the direct and label-free detection of ssDNA with an LOD as low as 20 nM. We have also demonstrated isothermal solid-phase recombinase polymerase amplification and detection of a low number of DNA copies (10 copies/uL). Furthermore, the solid-phase approach overcomes the limitations present in regular FPA-based analysis, where the production of by-product DNA sequences can hinder the final analysis, and, thus, requires the need for specific THF-based engineered primers. Therefore, traditional PCR primers can be used in the developed approach highlighting the simplicity and the generality of the system reported here.

Introduction

Isothermal solid-phase amplification and detection of genetic markers can be performed without labelling in real-time by utilizing both silicon microring resonators and solid-phase recombinase polymerase amplification. The technique was performed on a silicon microring resonator array chip with a microfluidic channel in contact to a temperature controller. For the solid-phase amplification, a hexyn-terminated primer of the target was directly attached to the surface of each microring resonator with a spotting device via click chemistry reaction. The amplified DNA was detected for each microring resonator by measuring the relative shift of the resonant wavelength during the DNA amplification on solid-phase. The probe spacing and surface density was optimised with different back-fillers to reach the best hybridisation conditions on solid-phase. Assay time lasted less than an hour at a constant temperature of 37°C, with high sensitivity and selectivity, avoiding the primer engineering associated to liquid-phase RPA.