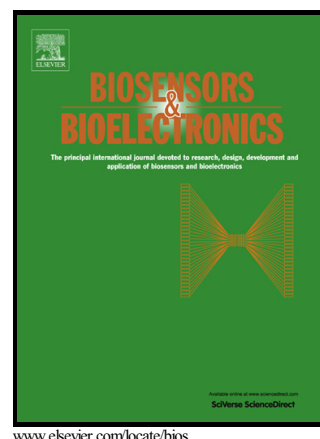


# Author's Accepted Manuscript

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# Real-time and label-free ring-resonator monitoring of solid-phase recombinase polymerase amplification

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## Abstract

In this work we present the use of a silicon-on-insulator (SOI) chip featuring an array of 64 optical ring resonators used as refractive index sensors for real-time and label-free DNA detection. Single ring functionalisation was achieved using a click reaction after precise nanolitre spotting of specific hexynyl-terminated DNA capture probes to link to an azido-silanised chip surface. To demonstrate detectability using the ring resonators and to optimise conditions for solid-phase amplification, hybridisation between short 25-mer single stranded DNA (ssDNA) fragments and a complementary capture probe immobilised on the surface of the ring resonators was carried out and detected through the shift in the resonant wavelength. Using the optimised conditions demonstrated via the solid-phase hybridisation, a 144-bp double stranded DNA (dsDNA) was then detected directly using recombinase and polymerase proteins through on-chip target amplification and solid-phase elongation of immobilised forward primers on specific rings, at a constant temperature of 37 °C and in less than 60 minutes, achieving a limit of detection of  $7.8 \cdot 10^{-13}$  M ( $6 \cdot 10^5$  copies in 50  $\mu$ L). The use of an automatic liquid handler injection instrument connected to an integrated resealable chip interface (RCI) allowed programmable multiple injection protocols. Air plugs between different solutions were introduced to prevent intermixing and a proportional-integral-derivative (PID) temperature controller minimised temperature based drifts.

**Keywords:** Ring resonator, Genosensor, Recombinase polymerase amplification.

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